Flexible filter feeders

The gelatinous zooplankton community in the Netherlands after the invasion of the ctenophore *Mnemiopsis leidyi*

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Chapter 1

Introduction

Lodewijk van Walraven

Jellyfish?

When most people think of jellyfish, they probably mean the large, conspicuous Scyphomedusae that sometimes wash up on the beach in massive numbers. These have attracted attention of naturalists for centuries. The occurrence of jellyfish on Dutch shores is already mentioned as early as the 16^{th} century. One of the oldest works on marine life in the Dutch language is the the 16^{th} century "*Visboek*" (Fish-book), written and illustrated by beachcomber and fisherman Adriaen Coenen in 1577–1581 (Egmond, 2005). In his book Coenen includes drawings of several species of jellyfish which he calls "*zeenetels*", sea-nettles in Dutch. Coenen mentions jellyfish as being "useless creatures" whose bad-smelling corpses were thrown on the beaches and who clogged up fishermen's net by sticking to the mesh, numbing the hands of the fishermen that had to remove them. On their origin he remarked "When they die they turn to water again, from which they originated".

Probably the earliest study of the seasonal patterns and abundance of jellyfish along the Dutch coast was by Van Deinse (1924) who counted them during beach visits from 1915–1917. The overall abundance trend of jellyfish (Fig. 1.1) showed highest abundances in June–October. The most abundant species found were *Aurelia aurita* and *Rhizostoma octopus*. Also here jellyfish are mentioned as causing problems, as in summer sea baths had to be closed when "enormous amounts of jellyfish occurred".

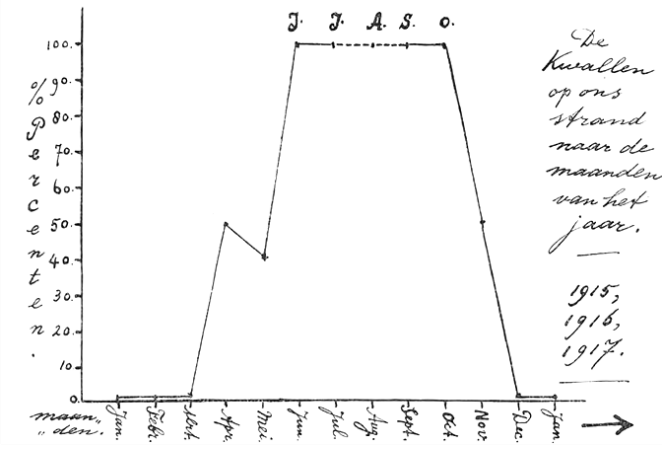

Figure 1.1: Seasonal abundance of jellyfish on Dutch beaches 1915–1917 with months Jan–Jan on the x-axis. Reproduced from Van Deinse (1924).

Nowadays most people still seem to think of jellyfish as nuisance species. Worldwide, concerns have been raised that jellyfish and jellyfish-related problems are increasing in response to various human-induced stressors (reviewed below). Are jellyfish abundances really increasing? And what will happen with projected climate change? This thesis aims to enhance our understanding of the role of jellyfish in Dutch coastal waters in general and that of an invasive species, the ctenophore *Mnemiopsis leidyi*, in particular. In the introduction, a short summary of gelati-

nous zooplankton feeding and life cycles is provided first, as there are major differences between taxa. An overview of the role of jellyfish in ecosystem structure and function is given. Following this, an overview of available knowledge on gelatinous zooplankton in Dutch coastal waters is presented and the outline of the thesis is described.

"Gelatinous zooplankton" or "gelata" are terms used to describe animals from many different phyla, such as medusae, ctenophores, molluscs, urochordates (salps) and worms. Gelatinous zooplankton are widespread and species of the different phyla can be found in waters ranging from the tropics to the poles (Haddock, 2004; Lucas and Dawson, 2014). Aside from having a (semi) transparent, fragile body, these animals differ in many ways from each other, and often have entirely different feeding and reproductive strategies. In this chapter the ecology of the three most abundant gelatinous zooplankton groups in Dutch waters is summarised.

Ctenophora

Ctenophora are the second oldest known multicellular animals to diverge, after Porifera. The alternative hypothesis that Ctenophora are the oldest known multicellular animals to diverge (Ryan *et al.*, 2013), which would imply several independent gains and losses of key metazoan characteristics, is currently not supported (Pisani *et al.*, 2015). 242 species of ctenophores have been described (Zhang, 2011). Ctenophores have a bi-radially symmetric body with a mouth on one polar end connected to a pharynx and stomach, and subsequently to the anal pore at the other polar end where the statocyst (sensory organ) is also located. Digested food is distributed through the body by means of eight meridional canals, along which the gonads lie in two bands. Also along the meridional canals lie the comb rows which are short, transverse plates of fused cilia arranged in succession. These comb rows are used for locomotion (Ruppert and Barnes, 1994).

Different feeding strategies are used (Costello and Coverdale, 1998). Cydippid ctenophores such as *Pleurobrachia pileus* are sit-and-wait predators and use long, branched tentacles containing colloblasts, sticky adhesive cells to which the prey adheres. Others such as the lobate ctenophore *Mnemiopsis leidyi* are filter feeders which use rows of cilia on auricules near the mouth to generate a feeding current which gets passed along surfaces lined with colloblasts. Beroid ctenophores such as *Beroe gracilis* are actively searching predators of other ctenophores. They either engulf the prey whole, or bite off parts (Fig. 1.2).

Ctenophores are simultaneous hermaphrodites, having both male and female reproductive organs at the same time. Fertilization takes place in the water. From the eggs a miniature ctenophore hatches. This means that unlike most scyphozoan species, ctenophores spend their entire life in the water column (holoplanktonic). Most species start as a spherical larvae with two tentacles (cydippid), later transforming in a different shape (Ruppert and Barnes, 1994). Their holoplanktonic lifestyle (Fig. 1.3) makes ctenophores more opportunistic than scyphozoans as they can more rapidly respond when favourable conditions occur.

Figure 1.2: *In vitro* photograph showing partial predation of *Mnemiopsis leidyi* by a much smaller individual of *Beroe gracilis*. Photo taken on 21/10/2011.

Scyphozoa

Most of the Scyphozoa, and all species that regularly occur in Dutch waters, have a life cycle involving both benthic and pelagic phases (Fig. 1.4). 228 species of Scyphozoa have been described (Zhang, 2011). The pelagic, adult medusa are of separate sexes. Males release sperm into the water column, which fertilises the eggs either in the water column or in brood pouches on the females' oral arms. From the eggs free-swimming planula larvae hatch, which in most species settle as a fixed polyp (scyphistoma) or as a cyst (planulocyst). The polyps can often change into cysts themselves or reproduce asexually in different ways reviewed in Arai (1997):

1. Bud off daughter polyps;
2. Form cysts, mostly at the pedal base (podocysts);
3. Strobilate: bud off tiny pelagic medusae (known as ephyrae) that can grow into adults and ultimately reproduce.

Cysts are "survival" stages which have a strong chitinous cuticle and very low respiration, enabling them to survive years without food and/or low oxygen levels.

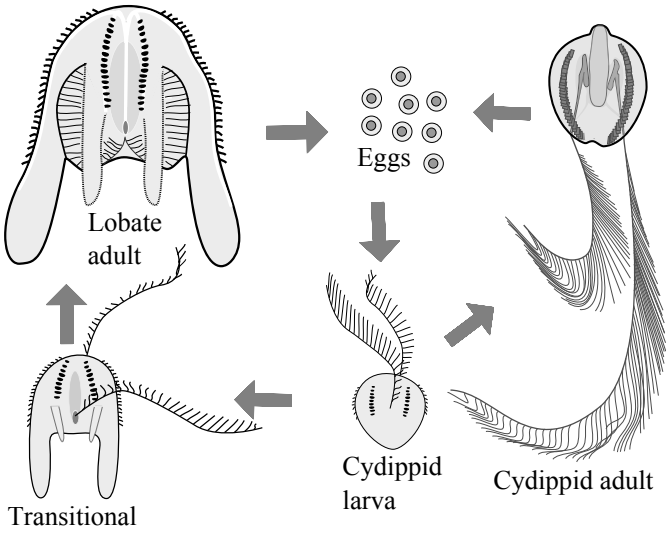

Figure 1.3: Simplified life cycle of lobate and cydippid ctenophores. Species depicted here are *Mnemiopsis leidyi* (left) and *Pleurobrachia pileus* (right, with adult *P. pileus* mouth-up).

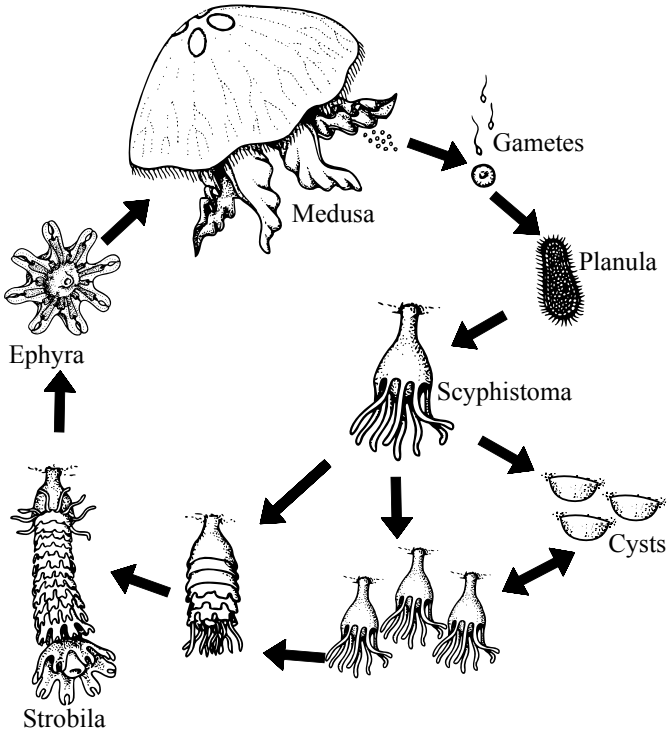

Figure 1.4: *Aurelia aurita* life cycle, modified from BIODIDAC (Morin and Houseman, 1995).

In general it is thought that scyphozoa have an annual life cycle; the adult medusae only reproduce once, after which they die (Arai, 1997). Some authors have suggested that the adults can overwinter, such as Russell (1970) who suggested that around the British Isles some species overwinter as adults in deeper waters.

Hydrozoa

Hydrozoa are a class of Cnidaria with 3500 species described (Zhang, 2011). The general life cycle of hydrozoa consists of a (usually benthic) polyp stage and (usually pelagic) medusa stage, similar to the scyphozoa, which both have separate sexes. The polyp stage reproduces asexually and often forms a colony, from which medusae are released, which grow and reproduce sexually (Fig. 1.5). From the fertilised egg a planula larvae is formed, which settles on a suitable surface and develops into a polyp (Russell, 1953).

However, almost all exceptions to this life cycle are also seen, as some species are benthic only with reduced medusa (gonophores) which never bud off, and others, such as *Velella velella* form pelagic colonies from which medusae are released. The life cycle of each species is described in Cornelius (1995a,b) for thecate hydroids and in Schuchert (2012) for athecates.

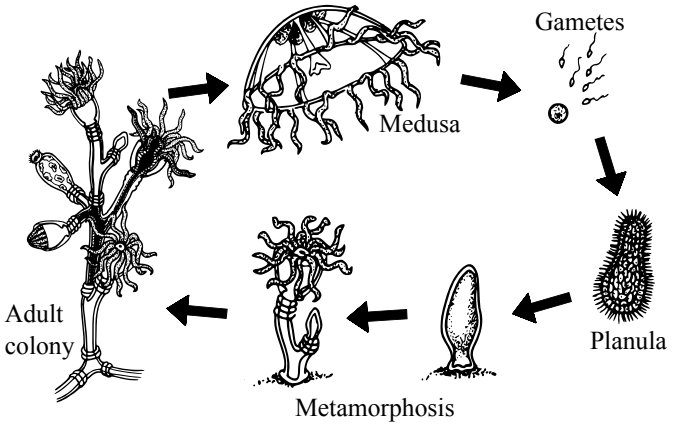

Figure 1.5: Life cycle of *Obelia* sp. representative for most hydrozoa with a benthic polyp stages, modified from BIODIDAC (Morin and Houseman, 1995).

The role of gelatinous zooplankton in ecosystem structure and function

Benefits of being gelatinous: jellyfish versus fish

Gelatinous zooplanktivores rely on tactile predation, whereas most zooplanktivorous fish species use visual predation as their main feeding method. Which method

is the most effective depends mainly on prey density and visibility. At high visibility and low prey density visual predators are more efficient feeders than tactile predators. As visibility decreases the feeding efficiency of visual predators decreases whereas that of tactile predators remains constant (Eiane *et al.*, 1999; Sørnes and Aksnes, 2004). Abundance of zooplanktivorous fish can be strongly correlated to water clarity (Aksnes, 2007). At low visibility tactile predation is more efficient than visual predation and changing visibility because of coastal water darkening (Aksnes *et al.*, 2009) or eutrophication (Haraldsson *et al.*, 2012) can lead to shifts in pelagic ecosystems from visual predators to tactile predators being the dominant zooplanktivores.

The primitive method of tactile predation employed by most gelatinous zooplankton species causes jellyfish to have a lower searching efficiency than fish, but this is compensated for by having a large body with a high water content, increasing prey contact rates. Because of this, jellyfish can have similar instantaneous prey clearance rates, respiration rates and potential for growth and reproduction as competing fish species (Acuña *et al.*, 2011). The efficiency of swimming in Scyphozoan jellyfish is increased by the use of passive energy recapture (Gemmell *et al.*, 2013). Prey encounter rates appear to be further optimised by switching between searching modes, from random walks to Lévy flight based on prey distribution (Hays *et al.*, 2012).

Global trends in gelatinous zooplankton

Scientific interest in the study of gelatinous zooplankton varied over the past centuries (reviewed in Haddock, 2004). The first peak in interest for gelatinous zooplankton occurred in the late 19^{th}–early 20^{th} century when many species of scyphomedusae, ctenophores and hydrozoans were described. In the mid 20^{th} century the focus of plankton studies shifted to large-scale quantitative sampling from fast-moving vessels. This contributed to a decrease in interest for gelatinous zooplankton as these are too fragile to be sampled with fast-moving samplers. Several gelatinous taxa are also difficult to preserve, further increasing the difficulty of studying these animals. In recent decades, from ca. 1980, scientific interest in gelatinous zooplankton increased again (Gibbons and Richardson, 2013), partly because the availability of new techniques to study them (Haddock, 2004) but also due to jellyfish-related disturbances of ecosystems such as the invasion of the Black Sea by the ctenophore *Mnemiopsis leidyi* (Purcell and Arai, 2001) described below.

Because of this varying interest, long-term time series on jellyfish are scarce. Also for the North Sea area, knowledge on the life cycles, population structure and long-term dynamics of the main gelatinous zooplankton species remains largely unknown and the underlying causes for the sudden arrival and disappearance of harmful blooms are poorly understood.

Eutrophication, climate change and overfishing are suggested to have created a situation in which gelatinous zooplankton species or "jellyfish" can proliferate and subsequently conquer an increasingly dominant role in ecosystems (Mills, 2001; Purcell, 2007, 2012; Jackson, 2008; Richardson *et al.*, 2009). As higher trophic

level predatory fishes are becoming increasingly overfished, fishing activity is shifting to species of lower trophic levels "fishing down the food chain" (Pauly *et al.*, 1998). When zooplanktivorous fish species decrease in abundance the predation pressure on the zooplankton is relaxed which favours other zooplanktivores such as jellyfish which can then become the dominant zooplanktivores in a process which is called "jellyfication" (Pauly *et al.*, 2002; Lynam *et al.*, 2011).

Eutrophication can lead to an increase in jellyfish abundance as well, by increasing food availability and decreasing competition by visual predators as described above. Eutrophication, as well as climate change, can lead to increasing events of hypoxia, and gelatinous zooplankton species have a higher tolerance to hypoxic conditions than fish (Grove and Breitburg, 2005; Richardson *et al.*, 2009).

Another important factor that can contribute to jellyfish blooms is the increase in available settlement sites for benthic polyps of hydromedusae and scyphomedusae. Artificial structures such as marinas, jetties, shipwrecks and oil rigs provide additional settlement substrates for the polyps (Duarte *et al.*, 2012; Lucas *et al.*, 2012).

Two syntheses of long-term datasets on jellyfish abundance worldwide indeed show an increase in abundance in recent decades. Brotz *et al.* (2012) analyse trends in aggregated datasets at the scale of Large Marine Ecosystems (LME's) and found that since 1950 in the majority of LME's jellyfish abundance has increased. Condon *et al.* (2012) performed a similar analysis by combining trends in 37 local jellyfish time series into a global "jellyfish index". They also find a slight increase in worldwide jellyfish abundance since 1970, but argue that this increase is small and has been mostly due to oscillations with an approximately 20 year periodicity caused by unknown factors.

Detriments and benefits associated with jellyfish

Regardless of whether there has been a global increase, in many areas gelatinous zooplankton are increasingly interfering with a wide range of human activities around the world (Hay, 2006; Richardson *et al.*, 2009). Many examples of jellyfish related problems are given in Purcell *et al.* (2007), Boero (2013), Gershwin (2013) and Lucas *et al.* (2014), with problems occurring mainly in the following sectors:

- Industry: Facilities that take in seawater such as power plants and desalinisation plants are experiencing problems by jellyfish clogging screens of water intakes. In several nuclear power plants reactors have had to be shut down after jellyfish clogged the cooling water intakes.

- Tourism: Most jellyfish are harmless, but several species have powerful stings and can cause severe injury or even death in humans (reviewed in Cegolon *et al.*, 2013). Reports of stinging incidents and the presence of jellyfish in an area can lead to tourists avoiding certain places.

- Fisheries: Gelatinous zooplankton can interfere with fisheries in several ways:

  - Directly by clogging fishing gear, spoiling catches or injuring fishermen who handle the animals.
  - Indirectly by competing for the same resource with fish species or by predating on the fish themselves, their larvae or eggs.

- Aquaculture: Hydroids can pose problems as fouling organisms on cages and nets. Both hydromedusae and scyphomedusae have been involved in mass killings of fish in aquaculture pens.

In face of all the "doom and gloom" stories associated with jellyfish, it is easy to forget that they also provide beneficial ecosystem services, as summarised in Doyle *et al.* (2014):

- When gelatinous zooplankton or their faecal pellets sink they transport carbon from surface waters to the seabed.
- In several ecosystems they act as keystone predator species or are important prey items for other organisms.
- Jellyfish themselves often provide habitats for other species, such as juvenile fish.
- Jellyfish are a traditional food in many Asian countries.
- Several novel compounds have been isolated from jellyfish, the most famous one being Green Fluorescent Protein (GFP) which is used to visualise processes taking place in living cells, such as protein expression.

Introduction of *Mnemiopsis leidyi*

First introductions

The first invasion of *Mnemiopsis leidyi* to waters outside its native range occurred in the 1980s in the Black Sea, most likely via ballast water of oil tankers. In the overfished and eutrophicated Black Sea *M. leidyi* spread very rapidly and formed large blooms (Vinogradov *et al.*, 1989). By competition for food and predation on larvae and eggs of zooplanktivorous fish species, which were already overfished, a collapse of the populations of these species occurred and subsequently of the fisheries that relied on them (Shiganova, 1998; Shiganova and Bulgakova, 2000).

Although the extent to which the *M. leidyi* invasion contributed to the collapse of pelagic fish stocks is still being discussed (Bilio and Niermann, 2004), the collapse had a substantial impact on fisheries in the region (Knowler, 2005). A few years after the natural predator of *M. leidyi, Beroe ovata*, was also introduced in the Black Sea, *M. leidyi* blooms decreased in frequency and magnitude. Eutrophication also decreased, and the Black Sea ecosystem seems to be recovering (reviewed in Kideys, 2002).

Second introductions

The first publications dealing with *M. leidyi* occurrence in European waters date from 2006, when reports were published of *M. leidyi* sightings in coastal waters off Sweden (Hansson and Kiørboe, 2006), Germany (Javidpour *et al.*, 2006) and the Netherlands (Faasse and Bayha, 2006; Tulp, 2006). Faasse and Bayha provided molecular identification of *M. leidyi*, by comparing its ITS-1 spacer region sequence with that of native *M. leidyi*, which differed only by a single base. Soon after, reports of *Mnemiopsis leidyi* occurrence in other areas appeared, and its presence in North-western European coastal waters has now also been reported from Denmark (Tendal *et al.*, 2007), Germany (in the North Sea (Boersma *et al.*, 2007) as well as in the Baltic Sea (Javidpour *et al.*, 2006)), Poland (Janas and Zgrundo, 2007) and Norway (Oliveira, 2007).

Even though its occurrence in North-western European waters was only confirmed in 2006, *Mnemiopsis leidyi* was most likely present there earlier, mistakenly identified as *Bolinopsis infundibulum*. Oliveira (2007) identified *M. leidyi* from pictures taken by recreational divers in Norwegian waters in August 2005. In Dutch waters *M. leidyi* was probably present much earlier than 2006; it is mentioned as early as 2002 (Holsteijn, 2002) and was present maybe even from in 1992 in Lake Grevelingen (Faasse and Ligthart, 2007). In a tidal fish trap on the island of Texel from 1988 onwards, unknown damaged ctenophores were observed mostly in November, but also in September and October in high densities. It is likely that these ctenophores were also *M. leidyi* (Royal NIOZ unpublished data and personal communication by Hans Witte, Royal NIOZ).

Mnemiopsis leidyi can be distinguished from *Bolinopsis infundibulum* mainly by the location of the termination of the oral lobes. In *B. infundibulum* the oral lobes terminate between the mouth and the apical sense organ (statocyst), while in *M. leidyi* they terminate near the apical sense organ, and span nearly the entire body length (Faasse and Bayha, 2006) and see (see Fig. B.2 in Appendix B).

Despite its presence and the influence it may have on ecosystems and especially on zooplanktivorous fish stocks (Purcell and Arai, 2001), until now no detailed study on aspects like population dynamics, abundance, predation on or competition with other zooplanktivorous organisms has yet been carried out for *Mnemiopsis leidyi* in Dutch coastal waters.

Source of the *M. leidyi* introduction

Population genetic analysis suggests that the *Mnemiopsis leidyi* introduction in Northern Europe was not related to the introduction in the Black, Asov and Caspian sea, but was a separate event. Two different studies share the same conclusion that North and Baltic sea *M. leidyi* originates from a North-western Atlantic (e.g. New England area) population and the Black Sea and surrounding seas *M. leidyi* originate from a more southern location such as the Gulf of Mexico (Reusch *et al.*, 2010; Ghabooli *et al.*, 2011).

North Sea current patterns suggest that the original introduction of *M. leidyi* occurred in the southernmost part of the North Sea, possibly in ports such as

Figure 1.6: *In vitro* photograph of *Mnemiopsis leidyi* collected in the western Dutch Wadden Sea.

Antwerpen or Rotterdam, from where it spread northwards. Genetic analysis however suggests the opposite, as genetic diversity was lower in the North Sea than in the Baltic Sea (Reusch *et al.*, 2010). The latter study however used few samples from the North Sea, and only from the Northern part of *M. leidyi*'s distribution range there. To further investigate this, samples from the Southern North Sea should be included.

Gelatinous zooplankton in Dutch Coastal waters: what is known?

The introduction of *Mnemiopsis leidyi* prompted a renewed interest in the gelatinous zooplankton of Dutch coastal waters. This section gives an overview of previous and current studies and non-scientific data sources on gelatinous zooplankton in this area. "Dutch coastal waters" refers to the estuaries and coastal areas, including the Dutch EEZ part of the North Sea and excluding the special municipalities overseas.

Species

Five species of ctenophores are known from Dutch coastal waters, of which one, *Mnemiopsis leidyi*, is invasive. All the other species discussed here belong to the

phylum Cnidaria; five species of Scyphozoa are observed regularly and a sixth, *Pelagia noctiluca*, is a rare migrant. Three species of Staurozoa have also been observed. Most species however belong to the Hydrozoa, of which 114 are considered native to the Dutch coastal area (Table 1.1). Nativity of the species is mostly based on the presence of benthic polyps; additional species enter Dutch waters as a medusae carried by currents from other areas.

Table 1.1: Overview of gelatinous zooplankton species found in Dutch coastal waters. Species status from the Dutch species database www.nederlandsesoorten.nl. Data def: Data deficient, status unknown.

Species		Status	Reference
Ctenophora			
Pleurobrachia pileus	O. F. Müller, 1776	native	Van der Veer and Sadée (1984)
Bolinopsis infundibulum	O. F. Müller, 1776	incidental	Holsteijn (2002)
Mnemiopsis leidyi	A. Agassiz, 1865	invasive	Faasse and Bayha (2006)
Beroe gracilis	Künne, 1939	native	Van der Veer and Sadée (1984)
Beroe cucumis	Fabricius, 1780	incidental	Holsteijn (2002)
Scyphozoa			
Aurelia aurita	Linnaeus, 1758	native	Van der Veer (1985)
Cyanea lamarckii	Pér. & Les., 1810	native	Van der Baan (1980b)
Cyanea capillata	Linnaeus, 1758	native	Van der Baan (1980b)
Chrysaora hysoscella	Linnaeus, 1767	native	Van der Baan (1980b)
Rhizostoma octopus	Linnaeus, 1758	native	Van der Baan (1980b)
Pelagia noctiluca	Forsskål, 1775	incidental	Van der Baan (1980b)
Hydrozoa			
Aequorea vitrina	Gosse, 1853	data def.	Ates (2005)
114 other species			see Vervoort and Faasse (2009)

Available information per water body

This part aims to give an overview of available knowledge on gelatinous zooplankton in Dutch coastal waters. Detailed observations along the Dutch coast (Verwey, 1942; van der Maaden, 1942) in the 1930s revealed clear patterns in seasonal occurrence of the scyphomedusae, with *Aurelia aurita* appearing from mid-May, *Cyanea lamarckii* from the end of May, *C. capillata* from the beginning of June, *Chrysaora hysoscella* in August and *Rhizostoma octopus* in September.

The most recent studies which focused on gelatinous zooplankton are from 1983, 1988 and 2007 for the Dutch Wadden Sea, Eastern Scheldt and North Sea, respectively (summarised in Table 1.2). In addition to scientific surveys, several long term volunteer monitoring programmes exist in the Netherlands (box 1.0.1) often spanning multiple decades. Data from these programmes offer valuable information, for example on the arrival of new species.

Table 1.2: Overview of quantitative zooplankton sampling programmes which included gelatinous plankton in Dutch coastal waters 1950 - present ordered by area and staring date.

area	location	period	frequency	gear	reference
Wadden Sea	Marsdiep basin	1981–1983	weekly	1.6 mm net	Van der Veer and Sadée (1984)
	Marsdiep basin	Spring 1982	incidental	plankton pump	de Wolf (1989)
	Ems-Dollard			1.6 mm net	
	NIOZ jetty (Marsdiep)	spring 1983	daily	1.6 mm net	Kuipers *et al.* (1990)
	Marsdiep	1960–present	daily	kom-fyke	Van der Veer *et al.* (1992)
Zeeland delta	Eastern Scheldt	1982–1988	weekly	63 µm pump	Bakker (1994)
				200 µm torpedo	Bakker and Rijswijk (1994)
North Sea	various	1950s–present	varying	Continuous Plankton Recorder	Batten *et al.* (2003)
	20 nm off Texel	1961–1966	weekly	2 mm net	Van der Baan (1967, 1980b,a)
	southern North Sea	summer 1977	incidental	300 µm torpedo 1.4 mm IK net	Seip and Seip-Ottema (1981)
	Frisian Front	1983,1986 1987,1990	incidental	300 µm torpedo 1.4 mm IK net	Kuipers *et al.* (1991)
	7 nm off Noordwijk	1984	daily in summer	50 µm net	Daan *et al.* (1985)
	German Bight	2004 and 2005	monthly in spring and summer	335 µm 500 µm net	Barz and Hirche (2007)
	Dogger Bank	June 2007	incidental	300 µm net	Frost *et al.* (2012)

North Sea

Long time-series of abundances of non-commercial fish and invertebrates in the North Sea spanning multiple decades are rare, and ones that are focused on gelatinous zooplankton such as ctenophores or scyphozoans are even rarer. The patchy nature of gelatinous zooplankton aggregations makes it difficult to take samples representative of the whole population (de Wolf, 1989), requiring a high sampling frequency. Because these kinds of sampling programmes are costly in terms of effort and money most of them are focused on commercial species of mainly fish. Luckily, these fish surveys often include some measure of the quantity of at least the large and conspicuous gelatinous zooplankters.

Increasingly, fish survey "by-catch" datasets are used to study long term trends in populations of gelatinous zooplankton (Bastian *et al.*, 2010).

Medusae

One "by-catch" dataset that has yielded valuable data on gelatinous zooplankton distribution in the North Sea was collected in June and July 1971-1986 during pelagic trawls of ICES 0-group Gadoid Surveys (Hay *et al.*, 1990). Later studies used this data to investigate the relationship between winter North Atlantic Oscillation Index (NAOI) and abundance of the scyphomedusae *Aurelia aurita*, *Cyanea lamarckii* and *Cyanea capillata* (Lynam *et al.*, 2004). This relationship differed between areas, ranging from negative in the southeastern North Sea to positive north of Scotland (Lynam *et al.*, 2005).

Other studies have looked at the frequency of nematocyst occurrence on Continuous Plankton Recorder (CPR) samples from CPR transects in the North Sea. The results from these studies are sometimes contradicting (Lynam *et al.*, 2010). Attrill *et al.* (2007) documented an increase in frequency of nematocyst occurrence in central North Sea CPR samples in the last decades that correlated positively with the North Atlantic Oscillation Index (NAOI), which contradicted the trend found in the trawl data by Lynam *et al.* (2004). A problem with this study and others using CPR data is that the nematocysts are not identified to species or even class level (Haddock *et al.*, 2008). This identification is very labour intensive, although some advances have been made (Baxter *et al.*, 2010). Recent studies using CPR data show an increase in Cnidaria frequency since the early 1980s in the North Atlantic as a whole (Gibbons and Richardson, 2009), and also separately in the north-east Atlantic and North Sea (Licandro *et al.*, 2010).

The most detailed North Sea dataset which includes gelatinous zooplankton is the Helgoland Roads meso- and macrozooplankton time-series, which samples zooplankton three times a week at a fixed station near the island of Helgoland in the North Sea (Greve *et al.*, 2004). These time series are a useful tool to study processes such as the timing of spawning or arrival of species at the beginning of the season, or to follow the arrival of new species and changes in species composition over the years. An analysis of *Pleurobrachia pileus* and *Beroe gracilis* phenology in the Helgoland Roads dataset revealed that the species shifted to an earlier appearance in the year, which was correlated with a stepwise increase in spring

seawater temperature (Schlüter *et al.*, 2010).

Juvenile and benthic stages

Unfortunately time series on juvenile stages of scyphozoa are almost absent. Van der Baan (1980b) studied the abundance of ephyrae at a single station in the North Sea close to the island of Texel (Table 1.3) and found seasonal patterns for some species, while others such as *Cyanea capillata* and *Rhizostoma octopus* were scarce or absent. The benthic stages of all species except *Aurelia aurita* have seldom to never been found in the North Sea or other Dutch coastal waters.

Table 1.3: Summary of data from the lightvessel "Texel" zooplankton sampling 1961 – 1966 by Gré van der Baan.

Species	Ephyrae present	Medusae peak
Aurelia aurita	early March - May, sometimes earlier	April - June
Chrysaora hysoscella	scarce, May - Aug, sometimes later	August/September
Cyanea lamarckii	usually November - May	two peaks, Dec. - Feb. and Jun. - Aug.
Cyanea capillata	unknown	very rare, later than *C. lamarckii*
Rhizostoma octopus	never	low numbers in Autumn

Wadden Sea

By comparison with the North Sea, little information is available on gelatinous zooplankton in the Wadden Sea. There have been two descriptive studies of the species composition of scyphomedusae in the Wadden Sea (Verwey, 1942; Wolff, 1983). In the early 1980s, the scyphomedusa *Aurelia aurita* (Van der Veer, 1985) and the ctenophores *Pleurobrachia pileus* and *Beroe gracilis* (Van der Veer and Sadée, 1984; Kuipers *et al.*, 1990) were present and abundant. *M. leidyi* has also been sighted in the Wadden Sea; the first confirmed records date from 2006 (Faasse and Bayha, 2006).

Zeeland estuaries

Quantitative data on gelatinous zooplankton in southern Dutch estuaries is only available for *Pleurobrachia pileus* in the Eastern Scheldt, which was monitored from 1982 – 1988 to document changes caused by the installation of a storm surge barrier in 1982–1986 (Bakker, 1994; Bakker and Rijswijk, 1994). Maximum *Pleurobrachia* densities generally occurred in May and ranged from 10 – 50 ind m^{-3}. There was no clear relationship between copepod and peak *Pleurobrachia* abundance, but often a decrease or levelling off of increase in copepods was observed after an increase in *Pleurobrachia* density, suggesting top–down control by the predator.

Semi−quantitative data is available for Lake Grevelingen and the Eastern Scheldt in the form of recreational scuba diver sightings (MOO project stichting ANEMOON http://oud.anemoon.org/result_moo/index.htm) for *Pleurobrachia pileus, Beroe gracilis* and most species of scyphozoa starting from 1994. Gittenberger (2008) used this data to investigate the seasonal patterns of *Mnemiopsis leidyi*, *Pleurobrachia pileus* and *Beroe gracilis* in the Eastern Scheldt and Lake Grevelingen. The chance of encountering *Mnemiopsis leidyi* during a dive was higher in Lake Grevelingen than in the Eastern Scheldt, and was highest in August in Lake Grevelingen and September − November in the Eastern Scheldt. The chance of encountering *P. pileus* and *B. gracilis* was highest in May and June, but both species were sighted year round.

Box 1.0.1 Volunteer monitoring programmes

There are several volunteer monitoring programmes targeting marine organisms on Dutch coasts:

- *The SMP project of the ANEMOON foundation monitors several four kilometre long stretches of beaches for shells and other stranded remains of marine organisms. Weekly/biweekly surveys have been carried out from 1978 onwards at a number of fixed locations along the Dutch coast. Starting at a single location in Katwijk-Noordwijk, in 1978 the number of locations increased to eight, the northernmost being on Texel and the southernmost at Neeltje Jans in Zeeland. Along a fixed transect parallel to the seashore abundance of animals is scored in abundance classes of orders of magnitude. See Gmelig Meyling and De Bruyne (1994) for details on locations and methods used and ANEMOON (2014) for the latest results.*
- *The MOO project of the ANEMOON foundation records sightings by SCUBA divers in the Netherlands, mainly in the Eastern Scheldt and Lake Grevelingen, since 1994 (Gmelig Meyling et al., 2013). On a standardised form divers record presence and absence of a selection of marine species. See Gmelig Meyling et al. (2013) for the latest results.*
- *Records of rare and interesting sightings by beachcombers are collected by the Strandwerkgemeenschap (SWG, 2014) and published in the Journal "het Zeepaard" since 1941.*

Changes in Dutch coastal waters over the last decades

Climate change

Recent work shows that the annual mean sea surface temperature in the western Wadden Sea has increased by 1.5 °C in the last 25 years. This increase was seen in each season, and was highest in winter; 1.9 °C (Van Aken, 2008a). In the North

Sea and the rest of the Dutch Wadden Sea a similar increase has been observed, which varies in magnitude (Van Aken, 2010).

Eutrophication

In the Wadden Sea riverine N and P influx increased gradually from 1935 until 1988, after which it decreased again, although values are still much higher than pre–1935 (van Raaphorst and de Jonge, 2004). Primary production doubled quickly in the 1970s, but decreased only slowly after the eutrophication decrease (Cadée and Cadée-Coenen, 2002).

Man-made structures

In Dutch coastal waters the amount of man-made structures such as sheets, revetments, oil and gas installations and wind farms with their associated scour protection has increased. For example, a large-scale coastal engineering project aimed at protecting the Delta area from flooding was completed in 1987 (Watson and Finkl, 1992), and in North-Western Europe, including the Dutch part of the North Sea, several wind farms have been constructed and more are planned (Breton and Moe, 2009). The increasing amount of man-made structures in coastal waters can lead to an increase in the amount of habitat available for polyps of scyphozoan and hydrozoa (Duarte *et al.*, 2012).

Fisheries

Stocks of pelagic zooplanktivorous fish such as herring *Clupea harengus* and sprat *Sprattus sprattus* have fluctuated greatly in the North Sea in the past 50 years (reviewed in Heessen *et al.*, 2005). The stock of herring collapsed in the early 1970s after heavy overfishing. After a period of closure to fisheries the stock increased again, followed by again a decrease in the mid 1990s. In recent years catches are increasing again.

Thesis outline

Present knowledge of gelatinous zooplankton in Dutch coastal waters is fragmented and scattered over many different sources. Apart from the Continuous Plankton Recorder programme and the NIOZ fish fyke, no programme has lasted longer than 5 consecutive years. Considering the three decade gap for which little data exists on gelatinous zooplankton in Dutch waters and the many changes observed in the area in the last decades, the main goal of this thesis is to investigate the present spatial and temporal distribution of gelatinous zooplankton species in Dutch coastal waters. How are these influenced by the many environmental changes observed in the area? Other questions asked are: What are the bottom-up and top-down controlling mechanisms of gelatinous zooplankton in Dutch coastal waters? What is the grazing pressure on the zooplankton community, and is there much competition with fish? How will projected climatic and other anthropogenically induced changes influence gelatinous zooplankton populations and their importance in Dutch coastal waters? As the most important change in the gelatinous zooplankton community has been the introduction of the invasive ctenophore *Mnemiopsis leidyi*, this species and its impact on the pelagic ecosystem of Dutch coastal waters is the main focus of the manuscript.

The first part of the thesis is focused on investigating changes in gelatinous zooplankton species composition, seasonal patterns and abundance in Dutch coastal waters. The first two chapters of this part are focused on the animals most people have in mind when they are talking about jellyfish: Scyphomedusae. In **Chapter 2** a unique 50-year time series of daily kom-fyke catches in the western Wadden Sea is used to investigate changes in phenology, abundance and species composition of Scyphozoan jellyfish and relate these to changing environmental conditions. In **Chapter 3** the distribution, species composition and population structure of jellyfish polyps is investigated by sampling a variety of different natural and artificial substrates in the southern North Sea and identify them using molecular markers.

For the next two chapters the focus is shifted to *Mnemiopsis leidyi*. In **Chapter 4** a first study on *M. leidyi* abundance and seasonal patterns in Dutch coastal waters shows that the species is present in high densities in the western Wadden Sea. Following this, in **Chapter 5** western Wadden Sea gelatinous zooplankton surveys from the 1980s are re-visited and compared with species composition, seasonal patterns and zooplankton grazing pressure in similar surveys performed in 2009–2012 in the same area, to investigate changes following the introduction of *M. leidyi*.

In the next part the phenotypical response of *Mnemiopsis leidyi* to different environmental conditions is studied to gain insight in the invasion success and possibilities to spread to new areas. In **Chapter 6** modelling and data analysis is combined to study the energy budget of *M. leidyi* over its full life-cycle using Dynamic Energy Budget (DEB) theory and literature data to investigate the response of different life stages of *M. leidyi* to changes in food and temperature. In **Chapter 7** the influence of salinity as a factor limiting the spread of *M. leidyi* in invaded areas is studied in a common-garden experiment where *M. leidyi* from two different sub-populations are raised at two salinity levels. We document the

first observation of a low salinity genotype of *M. leidyi* in Europe. In **Chapter 8** competition between *M. leidyi* and other zooplanktivorous species in the Wadden Sea is qualified by estimating diet overlap using Stable Isotope Analysis.

Finally, in **Chapter 9** the findings are discussed in a broader context. The role of Dutch coastal waters as a source of *M. leidyi* for western European waters is studied using a combination of hydrodynamic modelling and application of the Dynamic Energy Budget model parametrised in Chapter 6. The lack of monitoring of zooplankton in Dutch coastal waters is adressed and avenues for future research are suggested.

Chapter 2

Long-term patterns in 50 years of scyphomedusae catches in the western Dutch Wadden Sea in relation to climate change and eutrophication

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Abstract

Using a unique 50-year high resolution time series of daily kom-fyke catches, long-term patterns of scyphomedusae in the western Dutch Wadden Sea were analysed and related to changes in environmental conditions [eutrophication in the 1980s–1990s and recent climate change (increased water temperature)] in the area. Over the years, species composition and general pattern of appearance has remained the same: the first species that occurred in spring was *Aurelia aurita*, followed by *Cyanea lamarckii*/*C. capillata*. *Chrysaora hysoscella* and *Rhizostoma octopus* occurred from June–July onwards. All species appeared earlier in recent decades and first appearance and peak occurrence of *A. aurita* was in part inversely related to previous winter seawater temperature. Last occurrence of *C. hysoscella* was related to summer seawater temperature and the species is present longer in recent decades. Phenological relationships might have been decoupled since the seasonality of the phytoplankton bloom did not change. All species showed large interannual abundance fluctuations, with prolific years followed by sparse years. Peak catches of the coastal species *A. aurita* occurred in the late 1970s–early 1990s when eutrophication peaked, however, without a significant relationship with total nitrogen input into the area. Unlike for phenology, the patterns of mean abundance of any species did not show a relationship to climate change in the area. This might imply that population regulating mechanisms do not operate during the planktonic phase but during the sessile demersal polyp stages.

Introduction

Studies about jellyfish population dynamics are rare. One reason is their complex life cycle that includes a benthic and a planktonic phase: sexual reproduction produces free-swimming planula larvae that in most species settle as a semi-sessile polyp (scyphistoma). These polyps can form cysts or reproduce asexually by transverse fission and bud off tiny pelagic medusae (known as ephyrae) that grow and ultimately reproduce sexually (Arai, 1997). For many species, the location of these polyps in the natural environment is unknown. Gelatinous organisms are often fragile and more difficult to sample and preserve than crustaceans and fish (Haddock, 2004). Furthermore, they tend to occur in patches in the plankton, requiring a high spatial or temporal sampling resolution preferably of large volumes to obtain representative samples of abundance (de Wolf, 1989).

There is growing concern that gelatinous zooplankton mass occurrences are increasing in magnitude and frequency (Gibbons and Richardson, 2009) due to habitat modification, eutrophication, climate change and overfishing (Mills, 2001; Purcell, 2012). Long-term datasets are essential in determining whether such a worldwide increase in jellyfish biomass does occur (Condon *et al.*, 2012) and if so what the underlying mechanisms might be. However, such time series are scarce: one found an increasing trend in some large marine ecosystems (Brotz *et al.*, 2012), while in another study oscillations with a period of about 20 years were detected and only a weak increase overall (Condon *et al.*, 2013). The drivers behind this oscillating global trend are unclear but at least climatic variables seemed to be involved (Goy *et al.*, 1989; Thein *et al.*, 2013).

Occasionally, fish surveys include some measure of large and conspicuous gelatinous zooplankters and these 'by-catch' datasets have also been used (Hay *et al.*, 1990; Lynam *et al.*, 2011). A 16-year dataset of by-catches in a fish survey in the Irish Sea showed interannual variability in abundance of the jellyfish of which 68 % could be explained by hydro-climatic variables such as seawater temperature (Lynam *et al.*, 2011). Other studies have looked at the frequency of nematocyst occurrence in Continuous Plankton Recorder (CPR) samples. Lynam et al. (2011) analysed CPR transect data as well as fish survey catch data from the North Sea and found links of gelatinous zooplankton abundance with inflow of oceanic water and with the North Atlantic Oscillation Index. A problem with CPR data is that the nematocysts are not identified to species or even class level (Haddock *et al.*, 2008). Nevertheless, two other recent studies using CPR data show that Cnidaria frequency in the North Atlantic was cyclic (Gibbons and Richardson, 2009) and has increased in the north-east Atlantic and North Sea (Licandro *et al.*, 2010) since the early 1980s. This increase could not be linked to a single factor; instead both studies suggest that hydro-climatic changes are among the drivers. On the other hand, variation in a 20 year time series of abundance and phenology of *Aurelia aurita* and *Cyanea* spp. in Skagerrak could not be explained by environmental factors (Hosia *et al.*, 2014), despite the fact that experimental data on various North Sea scyphozoan jellyfish indicate that climate warming will benefit certain species (Holst, 2012a).

In this paper we present the first information on a 50-year time series of scy-

phomedusae for the western part of the Wadden Sea, a productive estuarine area of the North Sea bordering the Netherlands, Germany and Denmark. The data originate from an on-going high resolution long-term monitoring programme of daily kom-fyke catches focusing on epibenthic fauna in the western Dutch Wadden Sea (van der Veer et al., 1992; (van der Meer *et al.*, 1995; Campos *et al.*, 2010). Previous analysis have shown the reliability of these single trap observations in studying long-term trends: similar patterns in catches were found irrespectively of type of fish traps used or of location (Van der Veer *et al.*, 1992; van der Meer *et al.*, 1995).

The aim of the present study is to identify whether phenology and abundance of scyphomedusae in the western Wadden Sea has changed in response to climate change and eutrophication. Long-term trends in abundance, changes in phenology and possible common patterns (i.e. increased blooms) in scyphomedusae are identified and linked to environmental factors such as seasonal seawater temperature and salinity (Van Aken, 2008a,b) and changes in productivity in the area (Philippart *et al.*, 2010) caused by eutrophication in the 1980s–1990s (van Raaphorst and de Jonge, 2004). The analysis is based on count data only, similar as the analyses for shrimps by (Campos *et al.*, 2010). Since methodology, including mesh size, has been similar over the years, the minimum size caught by the kom-fyke will also have been similar over the years and therefore can be used to identify trends in appearance and abundance over time. The results will also be compared with long-term trends found in fish catch data (Van der Veer *et al.*, 2015).

Methods

Sampling

Kom-fyke

Since 1960, a kom-fyke trap has been operating at the entrance of the Marsdiep basin in the western Dutch Wadden Sea (Fig. 2.1). The kom-fyke consists of a 200 m-long and 2 m high leader which starts above the high water mark and ends in two chambers in the subtidal region with a mesh-size of 10 x 10 mm. For more details see Van der Veer *et al.* (1992). Fishing normally started in March–April and lasted until October. In winter the trap was removed because of possible damage by ice floes and from 1971 onwards no fishing took place during part of the summer because of fouling of the net and clogging by macroalgae. Fouling of the mesh panels perpendicular to the current direction leads to increased water resistance and hence stress on the wooden poles supporting the panels and to water flowing under the panels which can excavate the supporting poles. Clogging by large numbers of scyphomedusae also sometimes caused problems in summer.

Normally the kom-fyke was emptied every morning, except when bad weather prevented this. Pre-1973 when catches were small, the nets were sometimes emptied on alternate mornings. Here data for the period 1960–2010 were analysed, whereby the following catches were excluded:

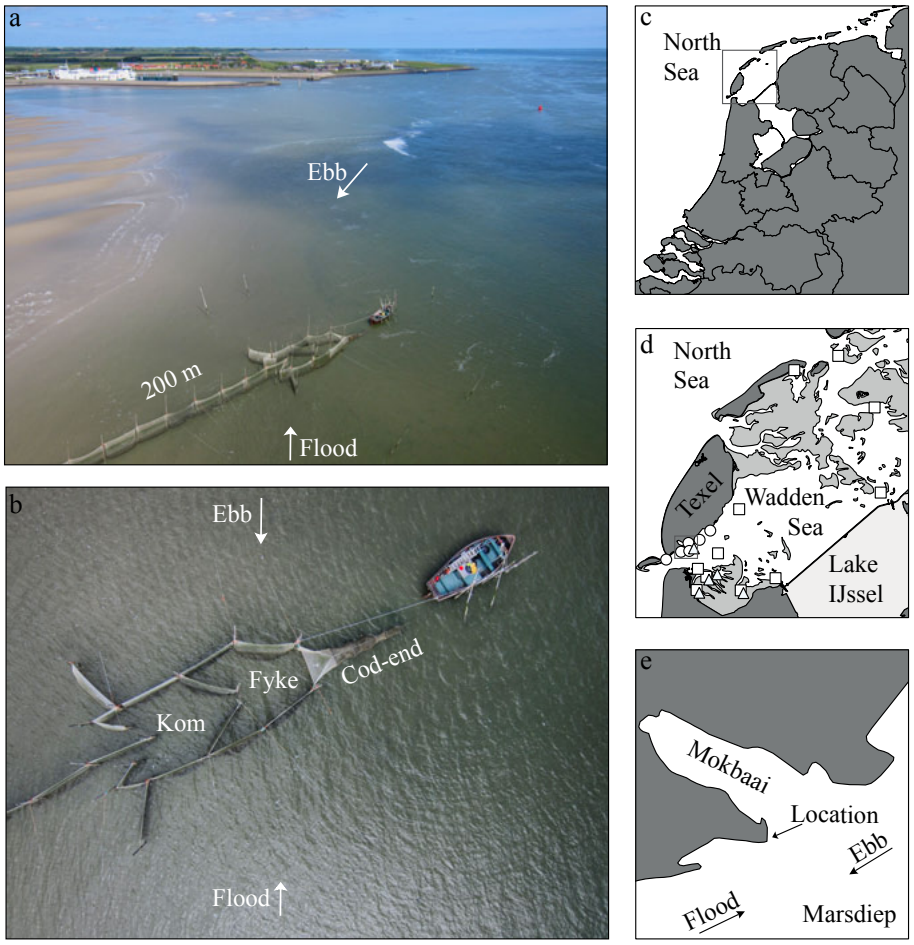

Figure 2.1: The NIOZ kom-fyke in the western Dutch Wadden Sea. Dark gray areas are land, light gray areas are intertidal flats. (a): aerial photograph showing the location of the kom-fyke; (b): aerial photograph showing the design of the kom-fyke. (c): location of the study area in the Netherlands; (d): location of the study area in the western Wadden Sea with the sampling stations of shipborne plankton net catches indicated: 1982 (triangles), 1983 (circles) and 2009 (squares); (e): location of the kom-fyke (arrow). Land is dark grey, intertidal areas are light grey. The kom-fyke system is situated at the end of a 200 m long leader. Directions of tides are indicated.

- a fishing duration longer than 48 h (329 records)
- a fishing duration shorter than 12 h (1 record)
- in case the gear was found to be damaged upon retrieval (loose mesh panels or tears) or clogged with debris (53 records).

In total, 6334 catch records were available for further analysis. All catches were sorted immediately and identified by species. Numbers of each scyphomedusae species were counted. For a detailed description of the method and fishing gear used, see Van der Veer *et al.* (1992) and van der Meer *et al.* (1995).

Plankton net sampling from ships

The impact of the summer interruption in fishing was investigated by comparing the patterns in the kom-fyke jellyfish catches with those of an independent year-round sampling programme for 1982, 1983 and 2009 at nearby stations in the same tidal basin (Fig. 1). These time series lasted respectively from February–December in 1982, from April–December in 1983 and from January–December in 2009. Oblique hauls were made with plankton nets made of polyamide plankton gauze (Monodur 2000, 2 mm mesh size) with an opening of 0.7 m^2, a length of 5 m, a porosity of 0.59 and a total surface area of 12 m^2. All data for all species was averaged per week for both the kom-fyke catches and boat stations and standardised by dividing the density or number by the maximum observed for each species in each year. For more details about these sampling programmes see Van der Veer (1985) for 1982, Kuipers *et al.* (1990) for 1983 and Van Walraven *et al.* (2013) for 2009.

Environmental covariates

In addition, abiotic information at different scales was collected:

- Sea surface temperature (SST) and salinity (SSS) near the kom-fyke were obtained from a nearby station (<500 m distance) (Van Aken, 2008a,b). From these daily SST and SSS data, indexes of seasonal seawater temperature and salinity were derived by calculating the mean SST and SSS for the following months: winter: January, February and March, spring: April, May and June, summer: July, August and September, autumn: October, November and December.
- Nutrient inputs (average per year in mol s^{-1}) of total nitrogen (total-N) and total phosphate (total-P) from Lake IJssel into the western Wadden Sea were taken as index of eutrophication of the area (van Raaphorst and de Jonge, 2004). Data for 1960–1994 were from van Raaphorst and de Jonge (2004) and for 1977–2010 from van Beusekom et al. (Van Beusekom *et al.* (2009); pers. comm.).

- The North Atlantic Oscillation winter index (NAOI) was used as an indicator of weather conditions on a broader scale [the Northern Hemisphere atmosphere, see Hurrell (1995)]. The December to March averaged difference between the normalised sea level pressure over Lisbon, Portugal and the normalised sea level pressure over South-west Iceland was taken as index from https://climatedataguide.ucar.edu/climate-data/hurrell-north-atlantic-oscillation-nao-index-station-based.

Data exploration

All data explorations and calculations were carried out in R version 3.0.2 (R Core Team, 2014). The data were explored following the protocol described by Zuur *et al.* (2010). Pairwise scatterplots of seasonal SST, seasonal SSS, total-N input, total-P input and NAOI hinted at collinearity between several covariates, which was confirmed by calculating the Variance Inflation Factors (VIFs) (Montgomery and Peck, 1992). Total P-input, was dropped because it was collinear with total N-input. Strong collinearity was also found between winter temperature and the NAOI. Previous work has shown that NAOI is correlated with winter sea surface temperature (Van Aken, 2008b) as well as precipitation in the river Rhine basin and consequently salinity in the western Wadden Sea (Van Aken, 2008a). Hence, the NAOI was excluded from further analyses. All remaining covariates had VIFs smaller than three. Cleveland dotplots revealed that there were no outliers (not shown). Data exploration showed clear differences in variation and timing between the four time series and therefore each species was analysed separately.

Data analysis

First appearance

Scatterplots of first occurrence versus time and versus the covariates showed clear non-linear patterns and therefore generalised additive models (GAM) were applied (Wood, 2006) using a Normal distribution with log-link. GAM assume homogeneity, normality and independence of residuals. To verify these assumptions, residuals of the models were inspected for temporal correlation using the autocorrelation function. Normality and homogeneity of variance of the residuals was also verified using histograms and plots of residuals versus fitted values. The GAM were applied with the gam functions in the mgcv package (Wood, 2006). Plots were created with the ggplot2 package (Wickham, 2009). For each species, the first day of occurrence in each year was determined. If this was the first day of fishing, that year was excluded from further analysis. To describe long-term changes in first occurrence we hypothesised a series of models (Table 1). The first model is a linear model which checks for a possible interaction between temperature and salinity:

The second model M1 hypothesised that winter temperature and salinity have additive effects on the date of first occurrence:

$$M1 : F_t = \alpha + f(Temperature_t) + f(Salinity_t) + \epsilon_t \quad (2.1)$$

where F_t is the date of first occurrence in year *t* ; *t* = 1960, ..., 2010. The terms $f(Temperature_t)$ and $f(Salinity_t)$ are smoothing functions of temperature (index of winter seawater temperature) and salinity respectively. The third and fourth model hypothesised that either temperature (M2) or salinity (M3) drives changes in first occurrence, and uses a single smoothing function for this covariate. It may well be that other factors are important and therefore we also considered models of the form:

$$M4 : F_t = \alpha + f(Year_t) + \epsilon_t \quad (2.2)$$

In this case $f(Year)$ is a smoother of time and represents the long-term trend in the data (model M4). Cross-validation was used to estimate the optimal amount of smoothing and a thin-plate regression spline was applied (Wood, 2006). The residual terms ϵ_t were assumed to be normally distributed and to have mean 0 and variance σ_2. All models were fitted and compared with each other using the corrected Akaike's Information Criterion (AICc) (Burnham and Anderson, 2002). The models were also compared with linear regression models fitted using the same covariates.

Peak occurrence

For each species in each year the day on which the highest number of medusae were caught was assumed to be the day of peak occurrence. To describe long-term changes in the timing of peak occurrence we repeated the analysis of first occurrence with the same models with F_t now being the date of peak occurrence in year *t* for each species.

Last occurrence

For each species in each year the day of the year on which the last medusae of a species were caught was assumed to be the day of last occurrence. To describe long-term changes in the timing of last occurrence we repeated the analysis of first occurrence with the same models with F_t now being the date of last occurrence in year t for each species and mean summer instead of winter values for temperature and salinity.

Duration of presence

To investigate whether the length of the period in which the medusae were caught each year changed over time, model M4 from the previous analyses was repeated with F_t now being the number of days between the first and last occurrence for each species.

Annual trends

To estimate trends and covariate effects over time, an index of annual abundance was calculated. First, seasonal occurrence of the various species was determined by estimating mean daily catch per month, for all years combined. These figures

were used to identify the months of peak occurrence for the various species. Next, for each species, the total catch in these months was considered as an index of abundance in that year. To compensate for differences in sampling effort over the years we used the base 10 logarithm of the number of sampling days as an offset in all models. Initial data exploration indicated that the temporal patterns in the four species were all different; therefore the abundance data from the various species was not combined. Data were analysed using Generalized Linear Models (GLM). As variances of the total catch per year were much higher than means we decided to use a negative binomial distribution. We hypothesised that the catch of each species was a function of the mean winter temperature of the area, mean winter salinity of the area and annual total-N input from Lake IJssel where

$$\mu_t = e^{intercept + Temperature_t + Salinity_t + Total-Ninput_t + epsilon_t} \quad (2.3)$$

and t=1960,...,2010. For *Rhizostoma octopus* mean spring temperature and salinity was used because data exploration showed that this species appeared and peaked several months later than the other species. Because ordinary GLM models suffered from residual autocorrelation we used a residual auto-regressive process of order 1 (AR1) in the predictor (Czado and Kolbe, 2004):

$$\epsilon_t = \epsilon_{t-1} + \gamma_t \quad (2.4)$$

Markov Chain Monte Carlo (MCMC) techniques were used in JAGS (Plummer, 2003) to estimate the parameters of the model. A burn-in of 150,000 iterations was used, with three chains and a thinning rate of 10. 9,000 iterations were used for the posterior distribution of each parameter. Diffuse normal priors were used for the regression parameters and a Half-Cauchy (25) prior was used for the parameter k in the variance of the negative binomial distribution (Zuur *et al.*, 2013). A Bayesian p-value was calculated to assess model fit; within each MCMC iteration data from a negative binomial distribution was simulated and was compared with the observed data.

Results

Environmental conditions

The environmental variables showed different temporal patterns (Fig. 2.2). Sea-water temperature and salinity varied considerably over the years; temperature showed an increasing trend in contrast to salinity. Nutrient loadings of total-N showed increasing loadings until the beginning of the 1980s followed by a decrease.

Species composition

The scyphomedusae recorded were: the moon jellyfish *Aurelia aurita* L.; two *Cyanea* species (the lion's mane jellyfish *Cyanea capillata* L. and the blue jellyfish *Cyanea lamarckii* Péron and Lesueur); the compass jellyfish *Chrysaora hysoscella*

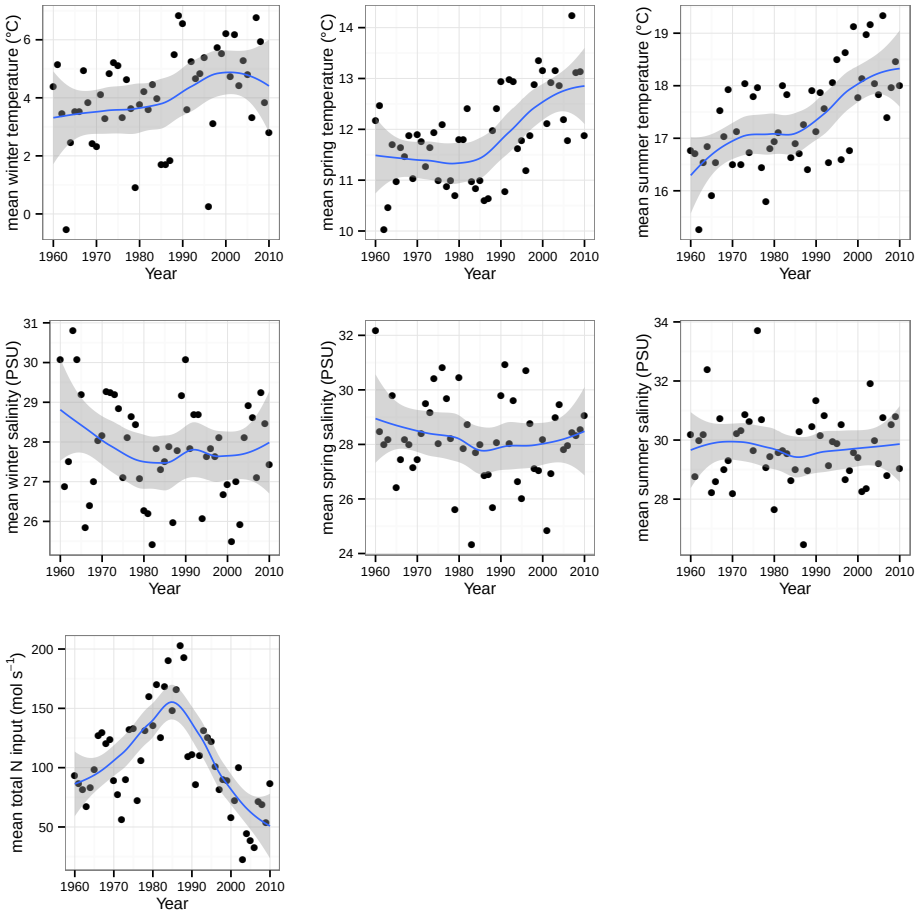

Figure 2.2: Trends in environmental conditions in the western Dutch Wadden Sea between 1960 and 2010. Seasonal means are for the following months: winter: January - March, spring: April - June, summer: July - September. For references see text. The solid line through the data is a LOESS smoother (LOESS span of 0.5). The shaded area is the 95% confidence interval of the smoother.

L. and the barrel jellyfish *Rhizostoma octopus* L. From the records, it was unclear whether the two *Cyanea* species were always identified correctly to species level; therefore they were combined into one category, *Cyanea* spp. Not all species were caught each year.

Seasonal distribution showed differences over time as well as among species. All species showed clear seasonal patterns (Fig. 2.3).

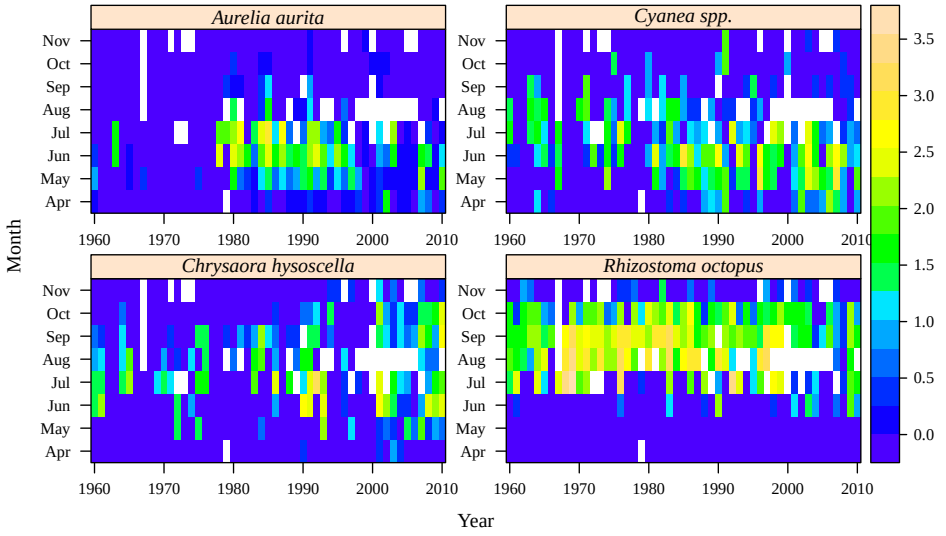

Figure 2.3: Contour plot of base 10 log (n + 1) values of mean daily catch (n day^{-1}), averaged per month for each year. Months in which there was no fishing are white.

Seasonal occurrence

Caveats

Several years had to be excluded for each species because first- peak- or last appearance happened on the first or last day of fishing (see below and Fig. 2.4). Peak- and last occurrence of 1967 was excluded because the kom-fyke was removed in the summer due to beach replenishment activities in the area. In the year 2000 there was limited fishing after the summer, and the same records were excluded for this year as well.

The impact of the summer interruption is presented for the years 1982, 1983 and 2009. The same species were present in the kom-fyke catches and in the plankton net catches (Fig. 2.5).

Jellyfish did not appear in the plankton net catches in the months before the fyke was operated (February and March in 1982 and January – March in 2009). There were also periods in which a species was caught in the kom-fyke, but not in the plankton nets, for example in *Cyanea* spp. after the summer interruption. First

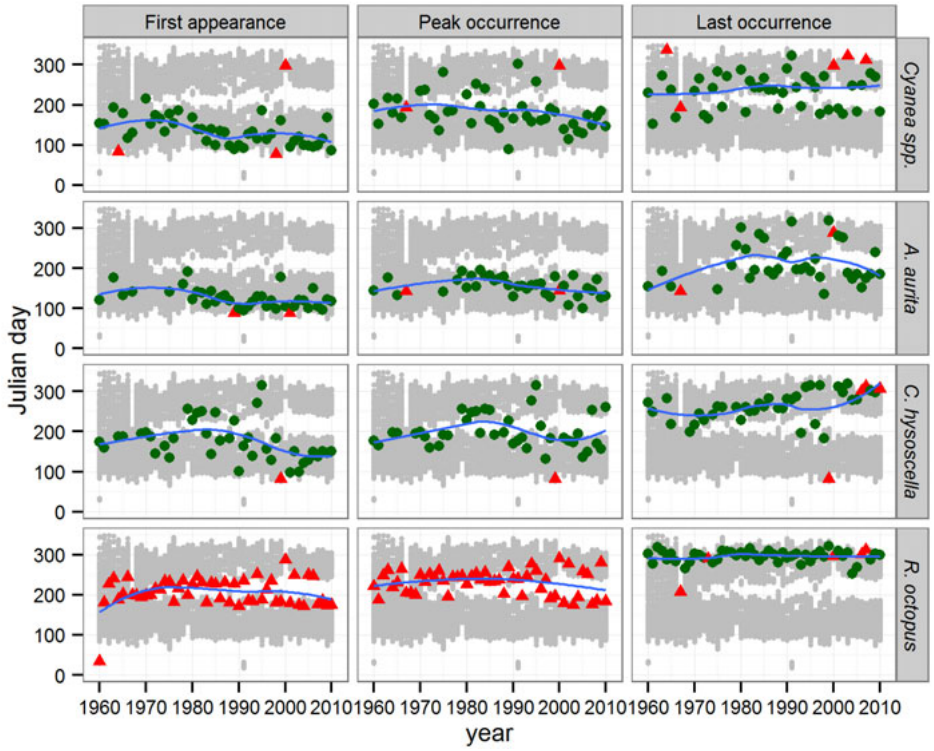

Figure 2.4: Date of first appearance, peak occurrence and last occurrence (day number) of *Aurelia aurita*, *Cyanea* spp., *Chrysaora hysoscella* and *Rhizostoma octopus* in the western Dutch Wadden Sea between 1960 and 2010. The solid line through the data is a LOESS smoother (LOESS span of 0.5). All days on which the fyke was operated are included as grey dots. Values that were excluded are marked as red triangles.

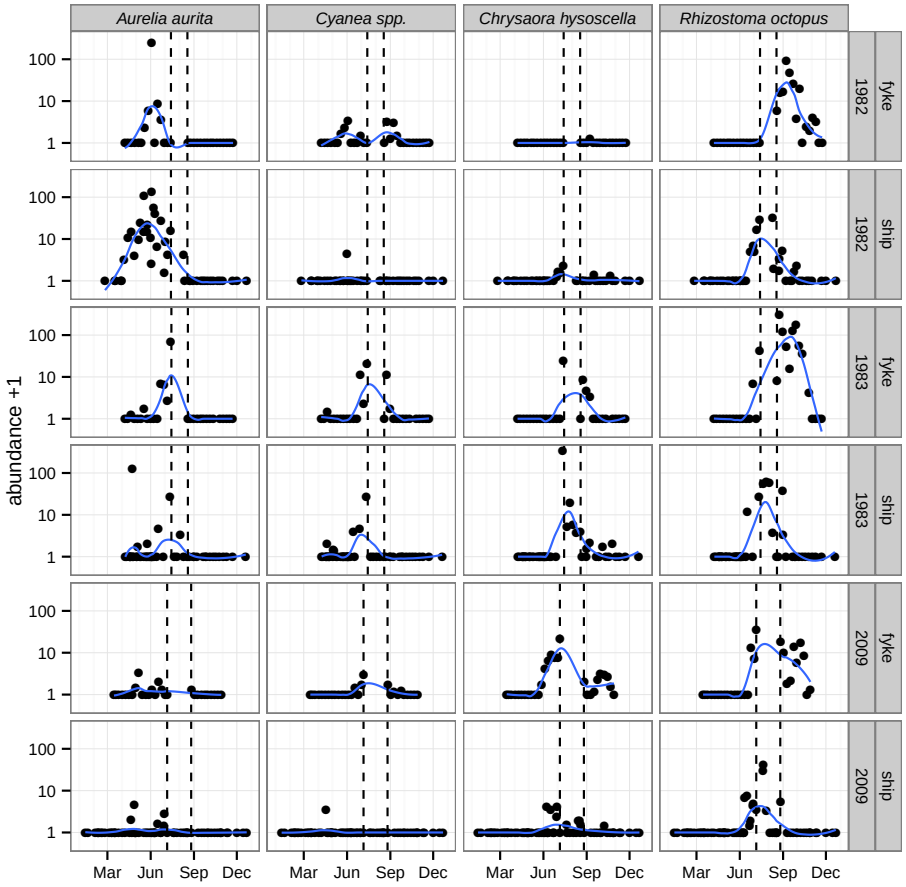

Figure 2.5: Abundance for shipborne plankton net (ship; n 1000 m^{-3}) for the kom-fyke (fyke; n day^{-1}). The summer period in which the kom-fyke was not operated lies between the vertical dashed lines.

appearance and peak occurrence of *Rhizostoma octopus* was later in the kom-fyke catches than in the plankton net catches and was often either at the beginning or at the end of the summer interruption, when also highest densities were observed in the plankton net catches (Fig. 2.5). Also, comments in the catch records from 1960–1971 suggest that the main species responsible for incidental clogging was *Rhizostoma octopus*. Because of these reasons, *R. octopus* was omitted from further analyses of first and peak occurrence. First appearance On average, the first species to appear in spring was *Aurelia aurita* (see Table 2.1), followed by the *Cyanea* spp. and *Chrysaora hysoscella*. In 18 years *A. aurita* was the first species to appear and in 4 years did the first appearance of the *A. aurita* and *Cyanea* spp. coincide. Since *A. aurita* was often absent in years, *Cyanea* spp. was the first to appear in 26 years. *C. hysoscella* was the first species to appear in only 4 years. Plots of date of first appearance showed that over time all species appeared earlier (Fig. 2.4). The model results are presented in Table 2.2. For *Aurelia aurita* model M1 had the lowest AICc value. This model had a significant ($p < 0.01$) negative linear smoother for temperature, and a non-significant smoother for salinity. There was a significant ($p < 0.05$) negative year effect. At the average winter salinity of 27.8 model M1 predicted a 6.8 day earlier appearance for each degree warmer winter temperature (Fig. 2.6). For *Cyanea* spp., there was a significant ($p < 0.01$) negative year effect. Winter temperature and salinity were not significant in the other models. AICc differences of the models with environmental covariates were very small. In *Chrysaora hysoscella* there was a significant ($p < 0.05$) year effect. None of the covariates in model M1, M2 and M3 were significant and as in *Cyanea* spp. there was little difference in AICc values of these models.

Table 2.1: Averages (with standard deviation in parentheses) of day of first appearance, peak- and last occurrence, catch at the day of peak occurrence and duration of presence for each species studied.

Averages	*A. aurita*	*Cyanea* spp.	*C. hysoscella*	*R. octopus*
First appearance (day)	126 (24)	134 (33)	176 (49)	-
Peak occurrence (day)	155 (23)	181 (43)	200 (41)	-
Catch at peak day (ind)	328 (367)	73 (116)	45 (71)	-
Last occurrence (day)	212 (49)	232 (42)	263 (35)	296 (14)
Duration (days)	84 (52)	100 (48)	95 (60)	-

Peak occurrence

In 25 years *Aurelia aurita* peaked first, in 16 years this was *Cyanea* spp. and in 7 years *Chrysaora hysoscella*. On average, *A. aurita* peaked on day 155, *Cyanea* on day 181 and *C. hysoscella* on day 200. The same models as for first appearance were fitted using the estimated day of peak occurrence (Fig. 2.4) as response variable (Table 2.3). In *Aurelia aurita* mean peak abundance was 328 ind day_{-1} and was very variable (sd 367 ind day_{-1}). Model M4 had the lowest AICc value. This model had a significant ($p < 0.05$) negative year effect. AICc values for model M1 and

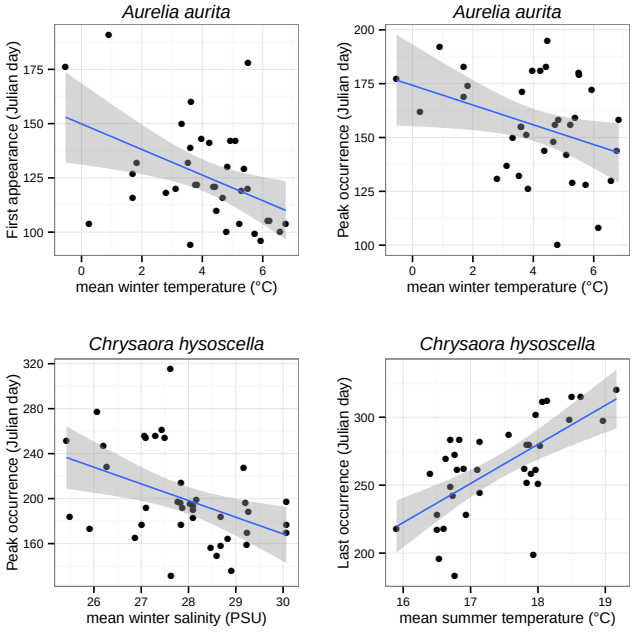

Figure 2.6: Top: Julian day of first appearance and peak occurrence of *Aurelia aurita* in relation to mean winter temperature (models M2). Bottom: Julian day of peak occurrence and last occurrence of *Chrysaora hysoscella* (bottom) in relation to mean winter salinity (model M3) and mean summer temperature (model M2) respectively in the western Dutch Wadden Sea. The solid lines through the data are the predicted values for each temperature or salinity, the shaded area is the 95% confidence interval.

Table 2.2: Overview of the results of the models describing timing of first appearance of the scyphomedusae species (*Aurelia aurita*, *Cyanea* spp. and *Chrysaora hysoscella*) in the western Wadden Sea. For each model we give the AICc, p-value and type of relationship (rel) with day of first appearance (-, negative, +, positive, bold: significant relationship; n.s., not significant)

species	model	type	variable	AICc	p	rel
A. aurita	M0	LM	Temperature	328	0.094	n.s.
			Salinity		0.284	n.s.
			interaction		0.068	n.s.
	M1	LM	Temperature	329	0.006	-
			Salinity		0.29	n.s.
	M2	LM	Temperature	328	0.008	-
	M3	LM	Salinity	334	0.443	n.s.
	M4	LM	Year	327	0.006	-
Cyanea spp.	M0	LM	Temperature	426	0.115	n.s.
			Salinity		0.044	+
			interaction		0.097	n.s.
	M1	LM	Temperature	426	0.104	n.s.
			Salinity		0.219	n.s.
	M2	LM	Temperature	425	0.096	n.s.
	M3	LM	Salinity	427	0.205	n.s.
	M4	LM	Year	411	<0.001	-
C. hysoscella	M0	LM	Temperature	432	0.406	
			Salinity		0.291	
			interaction		0.427	
	M1	LM	Temperature	430	0.253	n.s.
			Salinity		0.262	n.s.
	M2	LM	Temperature	429	0.222	n.s.
	M3	LM	Salinity	429	0.23	n.s.
	M4	GAM	s(Year)	426	0.032	-

M2 were equal, model M2 was selected as the best model as it was simpler and an ANOVA showed there was no significant difference between the two models ($p = 0.15$). Both models had a significant ($p < 0.05$) negative linear effect of temperature, and M1 had a non-significant smoother for salinity. Model M2 predicted a 4.7 day earlier appearance for each degree warmer winter temperature (Fig. 2.6). For *Cyanea* spp. mean peak abundance was 73 ind day-1 (sd 116 ind day-1). Model M4 had the lowest AICc value. None of the covariates were significant in any of the models. For *C. hysoscella*, mean peak abundance was 45 ind day-1 (sd 71 ind day_{-1}). Model M3 had the lowest AICc value. In model M1 and M3 there was a significant ($p < 0.01$) negative salinity effect (Fig. 2.6). None of the other covariates in the models were significant.

Table 2.3: Overview of the results of the models describing timing of peak occurrence of the scyphomedusae species (*Aurelia aurita*, *Cyanea* spp. and *Chrysaora hysoscella* in the western Wadden Sea. For each model we give the AICc, p-value and type of relationship (rel) with day of peak occurrence (-, negative, +, positive, bold: significant relationship; n.s., not significant)

species	model	type	variable	AICc	p	rel
A. aurita	M0	LM	Temperature	332	0.960	n.s.
			Salinity		0.516	n.s.
			interaction		0.949	n.s.
	M1	LM	Temperature	329	0.032	-
			Salinity		0.134	n.s.
	M2	LM	Temperature	329	0.036	-
	M3	LM	Salinity	332	0.158	n.s.
	M4	GAM	s(Year)	326	0.033	-
Cyanea spp.	M0	LM	Temperature	465	0.212	n.s.
			Salinity		0.22	n.s.
			interaction		0.871	n.s.
	M1	GAM	s(Temperature)	461	0.105	n.s.
			s(Salinity)		0.562	n.s.
	M2	GAM	s(Temperature)	459	0.128	n.s.
	M3	LM	Salinity	462	0.813	n.s.
	M4	LM	Year	457	0.031	-
C. hysoscella	M0	LM	Temperature	412	0.416	n.s.
			Salinity		0.134	n.s.
			interaction		0.425	n.s.
	M1	LM	Temperature	411	0.617	n.s.
			Salinity		0.006	-
	M2	LM	Temperature	416	0.518	n.s.
	M3	LM	Salinity	408	0.005	-
	M4	GAM	s(Year)	417	0.394	n.s.

Last occurrence

The day of last occurrence was very variable in *Aurelia aurita* (Fig. 2.4 and Table 2.1). In some years medusae were present after the summer as well, the latest in October. No significant relationships were found with year, temperature or salinity in any of the models (Table 2.4).

In *Cyanea* spp. last occurrence was also very variable and appeared to be centered around the summer gap in fishing. Again no significant relationships were found with year, temperature or salinity in any of the models.

Last occurrence in *Chrysaora hysoscella* appeared to shift to the end of the time series in the last two decades which was confirmed by a significant linear relationship between year and day number of last occurrence. Model M2 had the lowest AICc. In model M1 and M2 there was a highly significant positive effect of

summer temperature. For every °C increase in summer temperature the model predicted a 29 days later last occurrence (Fig. 2.6).

Rhizostoma octopus last occurrence was surprisingly constant near -but only in three years at- the last day of fishing. No significant relations were found with year, temperature or salinity in any of the models.

Duration

The length of the period between the first and last occurrence of medusae in the catches was very variable for all species (Fig. 2.7 and Table 2.1). *Aurelia aurita* was on average present for 84 days, but frequently for less than 40 days pre–1980. Their presence increased from 1960 to about 1980, hereafter it remained constant and showed a slight decrease in recent times but this trend was not significant (p=0.093). *Chrysaora hysoscella* was present for 95 days on average with a significant (p<0.01) non-linear year effect: the period in which it was present increased until 2010 and is now around double as long as in the 1970s. *Cyanea* spp. was present for 100 days on average with a significant non-linear increase (p <0.01) over time, especially from 1960 to 1985. Model validation showed that residuals were homogeneous and normally distributed and the auto-correlation function showed that the residuals were not auto-correlated.

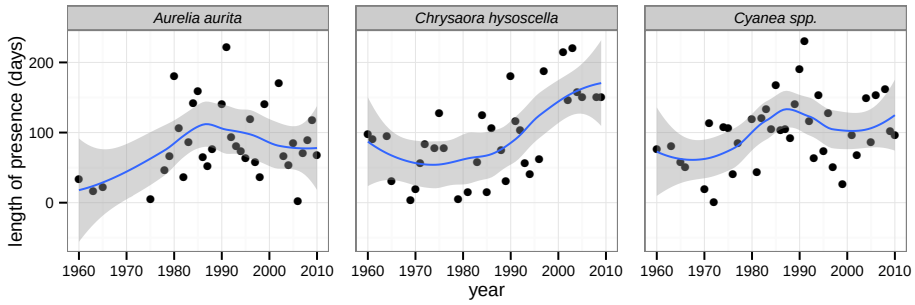

Figure 2.7: Length of period present (= number of days between the first- and last day of occurrence) between 1960 and 2010 for *Aurelia aurita*, *Cyanea* spp. and *Chrysaora hysoscella*. The solid line through the data is a LOESS smoother (LOESS span of 0.5). The shaded area is the 95% confidence interval of the smoother.

Annual trends

Patterns over time were irregular in all species, with no clear common pattern (see Fig. 2.3). *Aurelia aurita*, *Cyanea* spp. and *Chrysaora hysoscella* were absent for a number of years predominantly in the 1960s and 1970s. The months with highest catches were May–July for *A. aurita*, *Cyanea* spp. and *C. hysoscella*, therefore total catch in these months was used for further analyses.

The abundance index of the various species showed differences in absolute order as well as in pattern over time (Fig. 2.8). *Aurelia aurita* was the most abundant species, with mean daily catches often above 100 individuals and with a maximum of over 200 individuals in 1985. Abundances of *Cyanea* spp. and *Chrysaora hysoscella* were much lower: in all years the mean daily number of individuals caught always averaged below 30 and 60 individuals respectively. Abundance of *Rhizostoma octopus* was intermediate between that of *A. aurita* and the other species.

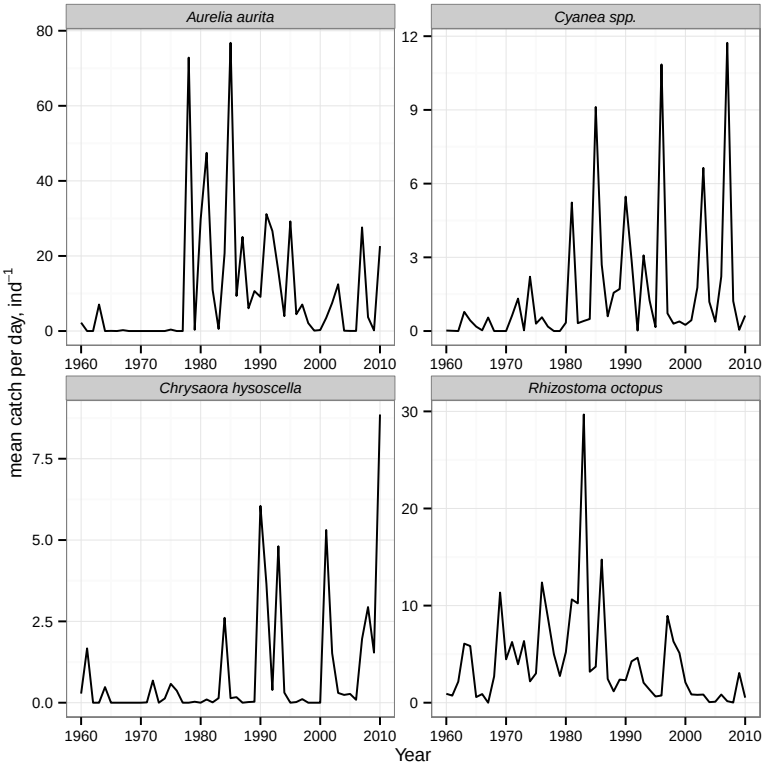

Figure 2.8: Index of annual abundance (mean daily catch; n day^{-1}) in months of peak abundance of the various scyphomedusae in the western Dutch Wadden Sea.

For all species the model with the covariates mean winter temperature of the area, mean winter salinity and annual total-N input from Lake IJssel was run. Model results (Fig. 2.9) indicated that none of the regression parameters were significantly different from 0. The auto-correlation parameters ρ indicated that there was significant, large auto-correlation for *Aurelia aurita*, but not for the other species. Model validation showed that there were no residual patterns, except for minimal residual auto-correlation in the *A. aurita* model. Bayesian p-values showed that the models were a good fit to the data.

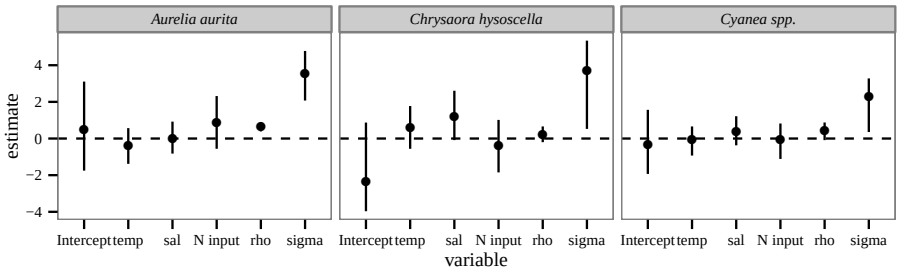

Figure 2.9: Model results of the Generalized Linear Model of total catch in the three months of highest catches for *Aurelia aurita*, *Cyanea* spp. and *Chrysaora hysoscella* in relation to mean temperature, salinity and total-N input from Lake IJssel. The figure shows the estimates and their 95% credible intervals. If the 95% credible interval covers the value of 0, a parameter is not significant. ρ determines the strength of the auto-correlation, σ is the dispersion parameter.

Table 2.4: Overview of the results of the models describing timing of last occurrence of the scyphomedusae species (*Aurelia aurita*, *Cyanea* spp., *Chrysaora hysoscella* and *Rhizostoma octopus*) in the western Wadden Sea. For each model we give the AICc, p-value and type of relationship (rel) with day of peak occurrence (-, negative, +, positive, bold: significant relationship; n.s., not significant)

species	model	type	variable	AICc	p	rel
A. aurita	M0	LM	Temperature	392	0.324	n.s.
			Salinity		0.362	n.s.
			interaction		0.337	n.s.
	M1	LM	Temperature	390	0.485	n.s.
			Salinity		0.373	n.s.
	M2	LM	Temperature	388	0.403	n.s.
	M3	LM	Salinity	388	0.638	n.s.
	M4	GAM	s(Year)	386	0.237	n.s.
Cyanea spp.	M0	LM	Temperature	433	0.672	n.s.
			Salinity		0.718	n.s.
			interaction		0.684	n.s.
	M1	GAM	s(Temperature)	429	0.633	n.s.
			s(Salinity)		0.17	n.s.
	M2	LM	Temperature	429	0.754	n.s.
	M3	GAM	s(Salinity)	426	0.144	n.s.
	M4	LM	Year	429	0.638	n.s.
C. hysoscella	M0	LM	Temperature	356	0.273	n.s.
			Salinity		0.35	n.s.
			interaction		0.376	n.s.
	M1	LM	Temperature	355	<0.001	+
			Salinity		0.305	n.s.
	M2	LM	Temperature	353	<0.001	+
	M3	LM	Salinity	372	0.109	n.s.
	M4	LM	Year	365	<0.01	+
R. octopus	M0	LM	Temperature	384	0.982	n.s.
			Salinity		0.967	n.s.
			interaction		0.99	n.s.
	M1	GAM	Temperature	378	0.135	n.s.
			Salinity		0.725	n.s.
	M2	GAM	Temperature	376	0.168	n.s.
	M3	LM	Salinity	379	0.491	n.s.
	M4	GAM	Year	378	0.824	n.s.

Discussion

The results show that over a 50-year period the seasonal occurrence of several species of scyphomedusae in the western Wadden Sea has changed in relation to climate change. In *Aurelia aurita* timing of first appearance and peak occurrence was negatively related to mean winter temperature. As winter temperatures have increased over the last decades the species is now occurring significantly earlier in the year. *Cyanea* spp. and *Chrysaora hysoscella* are also present significantly earlier in recent years but no relationship with winter temperature was found. *C. hysoscella* not only occurs earlier but is also present until later in the year in recent decades. The last occurrence of *C. hysoscella* species in the year was positively related to mean summer temperature. As a consequence the seasonal presence of the species is now twice as long in recent decades as in the first decades of the studied period.

All species showed large interannual abundance fluctuations, with prolific years followed by sparse years. Abundance of *Aurelia aurita*, *Cyanea* spp. and *Chrysaora hysoscella* were not related to any environmental factor included in this study although peak catches of the coastal species *A. aurita* occurred in the late 1970s–early 1990s when eutrophication peaked.

Caveats

A limitation of the kom-fyke data is that ephyrae and the smallest medusae are at least not quantitatively sampled by the 10 * 10 mm mesh size. Between strobilation and capture in the kom-fyke there is a period of weeks to months in which a species can be present in the water column but is not caught in the kom-fyke as indicated by the comparison with a sampling programme using a smaller mesh size in which the first appearance of *Aurelia aurita* and *Rhizostoma octopus* was several weeks earlier.

The main restriction of the time series became apparent during the summer period in which no fishing occurs. Especially for *Rhizostoma octopus* peak numbers of medusae are often observed during summer, limiting the importance of the kom-fyke for this species. For the other species the kom-fyke can be used for analysing the patterns in the period before and after the summer stop.

Species composition

The species composition has been constant over the last 50 years: 5 different species of scyphomedusae were recorded and those present in the 1960s were still present in recent years. The same species were recorded in the 1930s (Verwey, 1942; van der Maaden, 1942); so species composition seem to have been constant for almost a century. Only once was another species observed in the area: *Pelagia noctiluca* was caught in plankton samples taken in 1966 from the lightvessel "Texel" 10 nautical miles off the island of Texel (Van der Baan, 1967). Apart from *P. noctiluca*, the same species as found here were also caught in the lightvessel catches from 1961 – 1966 (Van der Baan, 1980b).

The distribution of the species found is overlapping but not the same. *Aurelia aurita* is a member of a cosmopolitan genus which can be found from the tropics to the polar regions but consists of several cryptic species (Dawson and Jacobs, 2001). *A. aurita* is considered a very common coastal and inshore species (Russell, 1970; Hay *et al.*, 1990). *Cyanea capillata* is a northern boreal species (Russell, 1970) but can be abundant in the southern North Sea as well. The distribution appears to be centered more offshore than that of *C. lamarckii* (Hay *et al.*, 1990). According to Russell (1970) *C. lamarckii* is a more southern species than *C. capillata*. It is an abundant species in the southern North Sea (Hay *et al.*, 1990; Barz and Hirche, 2007). *Chrysaora hysoscella* is a southern species of which the southern North Sea appears to be the northern distribution limit (Russell, 1970; Hay *et al.*, 1990). *Rhizostoma octopus* is a common species in western European waters which also appears to have a more southern distribution, although it is sometimes observed along the Scottish and Norwegian coast as well (Russell, 1970; Lilley *et al.*, 2009).

Long-term trends

Any trend analysis should be cautiously undertaken as some key processes may not be operating during the pelagic stage of the life cycle (Lucas *et al.*, 2012; Boero *et al.*, 2008) and the distribution of the benthic polyps is for most species unknown.

Detailed observations along the Dutch coast (Verwey, 1942; van der Maaden, 1942) in the 1930s revealed clear patterns in seasonal occurrence of the scyphomedusae, with *Aurelia aurita* appearing from mid-May, *Cyanea lamarckii* from the end of May, *C. capillata* from the beginning of June, *Chrysaora hysoscella* in August and *Rhizostoma octopus* in September. These authors identified the *Cyanea* species based on colour only however, which is not a unique characteristic of either species (Holst and Laakmann, 2014).

The medusae appearing in spring might not exclusively originate from ephyrae released a few months earlier, but could also be overwintering as *R. octopus* seems to do in the Irish and Celtic seas (Houghton *et al.*, 2007). Differences in timing between the species must be connected with the period of strobilation, which will be related to differences in life cycle, food conditions and temperature sensitivity (see for instance: Thein *et al.* (2013)).

The general pattern in seasonal peak occurrence corresponds with that in the 1930s, but the timing of first appearance is significantly earlier for all species: *Aurelia aurita* beginning of April, *Cyanea* spp. mid-April, *Chrysaora hysoscella* late June and *Rhizostoma octopus* from mid-July. Peak occurrence has decreased significantly over time in *A. aurita* and *Cyanea* spp. only. For *A. aurita* there was a significant negative relationship between winter temperature and first appearance; medusae appear earlier in the catches when winters were milder, either by being released earlier or by faster growth. A similar relationship was found between temperature and day of peak catches of this species. Peak occurrence of *C. hysoscella* was negatively related to salinity. The last occurrence of medusae in the kom-fyke did not change over time and was not related to any environmental covariate, except in *C. hysoscella* where medusae are occurring almost a month (29 days) longer for each °C increase in summer temperature. Even though *Aurelia*

aurita is occurring significantly earlier in the year, the length of the period in which it was present did not change over time. *Cyanea* spp. duration of presence doubled from 1960 until the 1980s after which it decreased slightly, then increasing again after 2000.

Relationship to climate change and eutrophication

The inverse relationship between winter temperature and date of first appearance and peak occurrence found for *Aurelia aurita* can be the result of several processes acting on the early life stages of the scyphomedusae. Firstly, strobilation could occur earlier. The mechanism of earlier strobilation at higher temperatures has been observed in cultured polyps of *A. aurita* from Taiwan (Liu *et al.*, 2009) and in *Aurelia labiata*, a sibling species of *A. aurita*, from Washington, USA (Purcell, 2007). Given the average temperature rise of the Wadden Sea by 1.5 °C over the last 25 years (Van Aken, 2008b), it might be expected that the various scyphomedusae will appear even earlier if temperatures continue to rise.

The second process that could contribute to earlier appearance would be increased growth rates. Growth rates will increase with increasing temperatures as long as the food supply can meet the increased demand and temperatures remain within the tolerance range of the species (cf. Freitas *et al.* (2007)). During the period of eutrophication of the western Wadden Sea from 1973 to 1991, the copepod *Temora longicornis* showed a strong increase in spring (March–June) (Fransz *et al.*, 1992). Unfortunately there is no data available on possible changes in food availability in the Wadden Sea area in more recent years.

All species expanded their seasonal occurrence by a shift to an earlier first appearance over time, but *Chrysaora hysoscella* is also present significantly longer in relation to mean summer temperature. *C. hysoscella* is a southern species of warmer waters with the southern North Sea as its northern distribution limit (Russell, 1970). In warmer summers the species might be able to survive longer in the area as temperatures stay within its tolerance range for an extended period. The negative relationship of timing of peak occurrence of *C. hysoscella* with mean winter salinity is difficult to explain. Salinity in the western Wadden Sea is influenced by freshwater input from rivers and coastal saline water input from the nearby North Sea (Van Aken, 2008a). A higher mean winter salinity could mean increased input of coastal water containing ephyrae/small medusae from elsewhere but this remains speculation.

The observed change in phenology of jellyfish to an earlier appearance in the year has not occurred in the whole marine pelagic community: during the last decades the timing of the phytoplankton bloom has remained the same (Philippart *et al.*, 2010). However, it remains unclear to what extent this implies that decoupling of phenological relationships has occurred, since in contrast to the North Sea (Edwards and Richardson, 2004) time series of zooplankton and fish larvae in the area are lacking. The annual data that are available for a few years shows that the timing of the zooplankton bloom is relatively constant but the magnitude is highly variable (Fransz *et al.*, 1992). In the neighbouring North Sea, a stepwise change in phenology of a gelatinous zooplankton predator (*Pleurobrachia pileus*) to earlier

appearance was observed, while the phenology of its food (calanoid copepods) showed no such change (Schlüter *et al.*, 2010). If the timing of the zooplankton bloom has remained the same in the Wadden Sea as well, earlier appearance of scyphomedusae could result in a mismatch between the abundance of predator and prey, as has been observed in other predator-prey relationships (Cushing, 1990). However, this earlier arrival could also create new matches between abundance peaks of prey and scyphomedusa predators that were previously temporally separated. Earlier work showed that flatfish larvae that migrate in their pelagic stage into the Wadden Sea mostly arrive before the annual increase in gelatinous predator abundance (Van der Veer, 1985). Earlier arrival of scyphomedusae would in this case mean increased predation risk on fish larvae.

Long-term trends in fish catches in the same kom-fyke during the same time period stresses the importance of environmental factors (Van der Veer *et al.*, 2015): the most important trend was a 10-fold decrease in total daily fish biomass from 1980 to the present of both pelagic and demersal species, whereby the most likely explanatory variables were thought to be habitat destruction in the coastal zone (sand dredging and beach nourishment, fishing) and large scale hydrodynamic circulation (the NAO winter index). The trend in fish catches did not correspond with the patterns observed in the various jellyfish species in this study.

With respect to annual trends in jellyfish abundance in the kom-fyke, different aspects play a role: the origin of the medusae (the location and distribution of the sessile polyps), long-term environmental changes in the western Wadden Sea in recent decades and the life stage were population regulation is operating. Differences in origins might imply different responses to the long-term changes in the western Wadden Sea. Over the last 25 years, there has been a gradual warming of the seawater (Van Aken, 2008b) and concomitant decline in salinity (Van Aken, 2008a).

Experimental work has shown that scyphistomae production of *Aurelia aurita*, *Cyanea lamarckii*, and *Chrysaora hysoscella* increases with warmer winter temperatures due to a longer strobilation duration, higher number of strobila per polyp or higher percentage of polyps strobilating (Holst, 2012a). When sufficient food is available, this should lead to increased number of medusae released. It is worthwhile to mention that the phenological response to environmental factors likely does not only differ between species, but also between populations adapted to different temperature regimes for example (Dawson *et al.*, 2015a).

Other environmental change in the area has been the eutrophication events that have affected the area, especially in the period 1970 to 1990. Riverine nutrient influx increased gradually from 1935 until 1988, after which it decreased again, although values are still much higher than pre-1935 (van Raaphorst and de Jonge, 2004). Primary production doubled quickly in the 1970s, but decreased only slowly after the eutrophication decreased (Cadée and Cadée-Coenen, 2002). The period with high jellyfish catches in the fyke (approximately 1978–1995) at least partly seems to match with the high eutrophication period. Both *A. aurita* and *R. octopus* catches decreased hereafter, though this is less clear in the latter because during summer —when *R. octopus* is most abundant— the kom-fyke is not operating as discussed above.

However, as none of the covariates in our models were significant, we could not confirm a relationship between eutrophication, water temperature or salinity and jellyfish catches in the kom-fyke. Variation in catches was very high, and this, combined with the need to add an autocorrelation structure to the model makes quantitative analysis of the data difficult. The significant temporal autocorrelation of the abundance of *A. aurita* and *R. octopus* (catch of jellyfish in one year is related to that in the previous year) might imply that density-dependent factors are operating, via the perennial, benthic phase of the life cycle.

To really understand the mechanisms underlying changes in phenology and abundance, detailed studies on benthic stages are essential. In line with Hosia *et al.* (2014), this study illustrates the importance of long-term datasets of gelatinous zooplankton in investigating possible drivers for changes in abundance and seasonal patterns. It also shows however that there are limits studying the pelagic medusa stage alone.

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Chapter 3

Where are the polyps? Molecular identification, distribution and population differentiation of *Aurelia aurita* jellyfish polyps in the southern North Sea area.

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Abstract

For many species of metagenic jellyfish the location of the benthic polyps is unknown. To gain insight in the distribution, species composition and population structure of scyphozoan jellyfish polyps in the southern North Sea area, polyp samples were collected from natural and artificial substrates (settling plates, marina floats and wrecks) at ten inshore locations in the Netherlands, seven offshore locations in the North Sea and in the Gullmar Fjord in Sweden. Polyps were identified to species level by sequencing both a fragment of 18S rDNA and a fragment of mitochondrial COI, and comparing these sequences to reference sequences available in GenBank and to newly obtained sequences from medusae collected in the area. All polyps sequenced did belong to *Aurelia aurita*. For this species, molecular diversity in mitochondrial COI was high, with 50 haplotypes among 183 polyps. Population differentiation was detected between the Dogger Bank and other – more coastal – locations, indicating extremely low connectivity. No significant differences were found between coastal samples. The location of polyps of *Cyanea capillata*, *Cyanea lamarckii*, *Chrysaora hysoscella* and *Rhizostoma octopus* in the study area remains unresolved.

Introduction

A variety of anthropogenic influences is suggested to contribute to increased gelatinous zooplankton blooms, such as climate change, overfishing, depletion of predators and increased habitat availability due to coastal and offshore engineering (Richardson *et al.*, 2009; Purcell, 2012; Duarte *et al.*, 2012; Lucas *et al.*, 2012). Especially the increasing availability of artificial substrates can contribute to an increase in jellyfish blooms for species with a benthic life stage (Duarte *et al.*, 2012).For example the introduction of a single 48 x 6 m pier caused an estimated 4.3 fold increase in the number of immature jellyfish (ephyrae) exported from a fishing port on the Inland Sea of Japan (Makabe *et al.*, 2014).

Many scyphozoan jellyfish species have a life cycle consisting of a sessile polyp stage and a free-swimming medusae stage. The male medusa releases sperm through its mouth into the water column. Fertilisation occurs in the female or in the water column (Schiariti *et al.*, 2012, and references therein). The fertilized egg develops into a free-swimming planula larva. The free-swimming planula larvae settle on hard substrate and metamorphose into sessile polyps called scyphistomae. Scyphistomae are a few millimeters in length (Holst *et al.*, 2007), inconspicuous and typically inhabit shaded environments often underneath horizontal surfaces of rocks and shells (Pitt, 2000). They settle on a wide range of artificial substrates such as breakwaters, marina pontoons, plastic waste and aluminum cans (Holst and Jarms, 2007; Purcell, 2009; Duarte *et al.*, 2012). Polyps are most abundant in the early stages of colonisation of substrates (Lindeyer and Gittenberger, 2011; Makabe *et al.*, 2014).

Polyps of most species propagate asexually. A polyp can live for several years (Arai, 1997). The transition from polyp to medusae is also a way of asexual reproduction; immature medusae (ephyrae) are released into the water column by strobilation. One polyp can produce as many as 40 ephyrae during each strobilation event (Lucas, 2001). Asexual reproduction and the perennial duration of the polyp stage can result in apparently unregular and unpredictable patterns in abundance of medusae (Boero *et al.*, 2008). Due to the small body sizes and cryptic lifestyle, these sessile stages of many jellyfish species are often unnoticed and their location unknown. Because polyps are the source of metagenic scyphozoan blooms (Arai, 1997), knowledge of their distribution is key to understanding and predicting the response of scyphozoan populations to factors such as climate change (Mills, 2001) and the increasing availability of artificial substrate (Duarte *et al.*, 2012).

In areas such as the southern North Sea where natural hard substrate is absent or scarce (ICES, 2016) artificial structures can be seen as “oases of marine biodiversity” because they offer hard substrate where normally only soft bottoms occur (Lengkeek *et al.*, 2013b; Schrieken *et al.*, 2013). With approximately 600 oil and gas installations, 2,584 km^2 wind farms and 27,000 wrecks present in the North Sea area, the availability of artificial substrates is significant (ICES, 2016; Coolen *et al.*, 2015b). Similarly, in nearshore areas, structures such as marinas offer additional settlement substrates. In the southern North Sea and bordering Dutch coastal waters, scyphozoan polyps have been found on several types of artificial structures in several locations. Lindeyer and Gittenberger (2011) found

scyphistomae on PVC settling plates, suspended at 1m depth in marinas and ports in various locations in the Eastern Scheldt and Lake Grevelingen. Polyps have also been found on other hard substrates in the same areas (De Kluijver and Leewis, 1994; Gmelig Meyling *et al.*, 2013). In the North Sea polyps are found on artificial reefs (van Moorsel, 1993), on various shipwrecks (Waardenburg, 1987; van Moorsel *et al.*, 1991; Leeuwis and Waardenburg, 1991; van Moorsel and Waardenburg, 1992b; Hiscock *et al.*, 2010; Lengkeek *et al.*, 2013a), oil platforms (Guerin, 2009) and wind farm foundations (Vanagt and Faasse, 2014). In most of these studies the polyps are assumed to belong to *Aurelia aurita*.

Medusae of five species of scyphomedusae are commonly found in most Dutch coastal waters: the moon jellyfish *Aurelia* sp., the compass jellyfish *Chrysaora hysoscella*, the lion's mane jellyfish *Cyanea capillata*, the blue jellyfish *Cyanea lamarckii* and the barrel jellyfish *Rhizostoma octopus*. These are found in the North Sea (Van der Baan, 1980b; Hay *et al.*, 1990; Barz and Hirche, 2007), Eastern Scheldt estuary (Bakker, 1994) and Wadden Sea (Van der Veer, 1985; Van Walraven *et al.*, 2015). In the saline lake Grevelingen all species except *C. capillata* are found (Gmelig Meyling *et al.*, 2013). Except for *Rhizostoma octopus*, these species have a metagenic life cycle where fertilization occurs in the female. Embryonic development takes place inside specific brood pouches in the oral arms in the *Cyanea* species and in *Aurelia*. In *Chrysaora hysoscella* planulae develop inside the gonads and in *Rhizostoma octopus* planulae develop externally (Russell, 1970; Holst *et al.*, 2007).

Experimental work has shown that the polyps of these five scyphozoan species can not be identified based on morphological features alone (Holst, 2012b). None of the studies mentioned above used molecular methods to identify the polyps found, nor identified them by inducing them to strobilate and identifying the ephyrae, so it is possible that species other than *Aurelia aurita* were present. Therefore, the main goal of this study is to identify to species level scyphozoan polyps found in these locations using a slowly evolving marker (nuclear 18S rDNA) and a fast evolving marker (mitochondrial COI) for molecular species identification.

Population subdivision is a typical find in population genetic studies of jellyfish (e.g. in Dawson *et al.*, 2005; Ramšak *et al.*, 2012; Lee *et al.*, 2013). Connectivity between areas tens to hundreds of kilometers apart may be extremely low (Dawson *et al.*, 2015b), but apparent panmixis up to large geographic scales has also been observed (e.g., Stopar *et al.*, 2010; Miller *et al.*, 2012; Dong *et al.*, 2015). Genetically different populations of the same species can exhibit differences in factors such as the timing and magnitude of medusae blooms (Dawson *et al.*, 2015b). Knowledge of the genetic structure of jellyfish population can thus be important in predicting when and where scyphozoan jellyfish blooms occur.

To date, studies on population structure of metagenic scyphozoa have sampled the mobile medusae, rather than the sessile polyps. Medusae can disperse over long distances during their life, while polyps are typically fixed. The second goal of this study was to investigate whether population differentiation exists in scyphozoan polyps in the southern North Sea area. When there is population differentiation between polyp populations in the area, it could be possible for example that the phenotypical response to changing environmental conditions could differ for polyps

of the same species in different areas.

Methods

Specimen collection

Medusae of the five dominant species present in the area were collected from net tows and beaches at several locations in the southern North Sea area (Table 3.1 and Figure 3.1). As reference material for species identification using molecular markers a piece of bell margin was clipped and stored in 2 ml Eppendorf cups filled with 96 % EtOH. Polyps were collected in various ways: from artificial setting plates, from floats in Dutch ports and marinas, and by scuba diving from hard underwater substrates.

Settling plates were deployed at 1 m depth in various Dutch marinas and ports as part of an ongoing programme aimed at monitoring the presence of invasive species (the SETL programme of the ANEMOON foundation). The plates consisted of a 14 x 14 cm 0.5 cm thick PVC of which the bottom side was sanded to create a rough surface. Plates were attached to a standard brick (Figure 3.2) and deployed at a standard depth of 1 m and are periodically checked as described in Lindeyer and Gittenberger (2011). Between December 2012 and May 2013 settling plates were removed from the water and, submerged, checked for the presence of scyphozoan polyps by eye on eight different locations. If present, a minimum of two polyps per plate were removed with tweezers and stored in 2 ml Eppendorf cups in seawater. Using a binocular microscope the polyps were cleaned of debris and remains of the substrate, after which they were stored individually in 2 ml Eppendorf cups filled with 96 % EtOH.

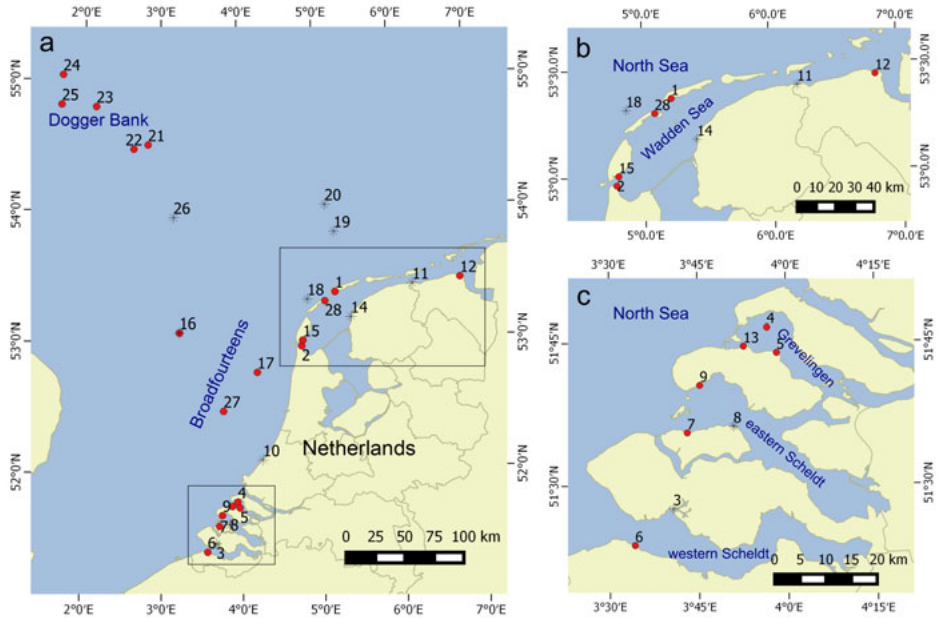

Figure 3.1: Overview of sampled locations. Polyps were found on locations indicated with red filled circles and not found on locations indicated with stars. Numbers correspond to the numbers in table 1. (a) whole studied area (b) Wadden Sea (c) Zeeland. The Skagerrak location is not shown.

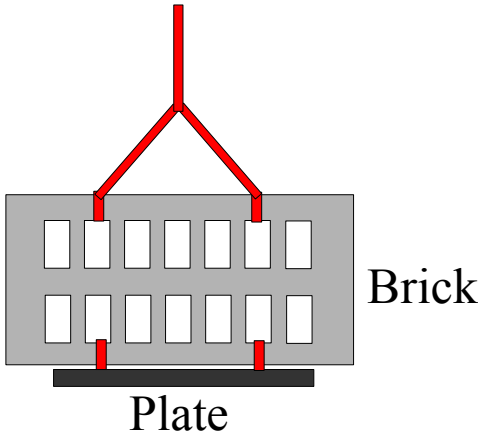

Figure 3.2: The SETL plate design. The PVC plate is attached to the brick with zip-ties. The brick is 21.2 cm long, 9.7 cm high and 5.2 cm wide.

Table 3.1: Overview of sampled locations. n = number of sequences obtained, d = depth in meters.

nr	location type	place	area	n	d (m)	polyps found	coll. type	date	deg. N	deg. E
1	Marina	West-Terschelling	western Wadden Sea	3	1	Yes	Float	05/07/2010	53.3651	5.2216
2	Marina	Den Helder	western Wadden Sea	9	1	Yes	SETL	14/12/2012	52.9616	4.7804
3	Harbor	Vlissingen	western Scheldt, Zeeland		1	No	SETL	20/12/2012	51.4598	3.6782
4	Harbor	Hompelvoet	Grevelingen, Zeeland	10	1	Yes	SETL	18/03/2013	51.7761	3.9464
5	Harbor	Bommenede	Grevelingen, Zeeland	10	1	Yes	SETL	18/03/2013	51.7317	3.9729
6	Marina	Breskens	western Scheldt, Zeeland	15	1	Yes	SETL	09/04/2013	51.3958	3.5704
7	Marina	Kamperland	eastern Scheldt, Zeeland	12	1	Yes	Float	10/04/2013	51.5923	3.7190
8	Marina	Colijnsplaat	eastern Scheldt, Zeeland		1	No	Float	10/04/2013	51.6033	3.8496
8	Marina	Colijnsplaat	eastern Scheldt, Zeeland		1	No	SETL	10/04/2013	51.6033	3.8496
9	Marina	Burghsluis	eastern Scheldt, Zeeland	4	1	Yes	Float	10/04/2013	51.6755	3.7553
10	Marina	Scheveningen	central North Sea		1	No	Float	29/04/2013	52.0960	4.2660
11	Marina	Lauwersoog	western Wadden Sea		1	No	Float	01/05/2013	53.4099	6.2113
12	Harbor	Eemsmond	eastern Wadden Sea	7	1	Yes	Float	01/05/2013	53.4448	6.8246
13	Oyster reef	Den Osse	Grevelingen, Zeeland	2	20	Yes	SCUBA	05/05/2013	51.7434	3.8796
14	Harbor	Harlingen	western Wadden Sea		1	No	SETL	11/05/2013	53.1707	5.4133
15	Harbor	t Horntje	western Wadden Sea	3	1	Yes	SCUBA	20/05/2013	53.0056	4.7964
16	Wreck	Russian submarine	Broadfourteens, North Sea	4	33	Yes	SCUBA	03/07/2013	53.0718	3.2326
17	Wreck	Vinca Gorthon	Broadfourteens, North Sea	10	20	Yes	SCUBA	05/09/2014	52.7662	4.2128
18	Wreck	Vittorio Z	Frisian Front, North Sea		14.4	No	SCUBA	06/09/2014	53.3142	4.8662
19	Wreck	Unknown	Frisian Front, North Sea		33.7	No	SCUBA	06/09/2014	53.8263	5.2267
20	Wreck	Healdton	Frisian Front, North Sea		37.4	No	SCUBA	07/09/2014	54.0342	5.1198
21	Wreck	wreck nr. 59695	Dogger Bank, North Sea	5	33.3	Yes	SCUBA	09/09/2014	54.5035	2.8293
22	Wreck	Ocean Prince	Dogger Bank, North Sea	14	29.4	Yes	SCUBA	09/09/2014	54.4712	2.6440
23	Wreck	wreck nr. 70502	Dogger Bank, North Sea	10	27	Yes	SCUBA	10/09/2014	54.7952	2.1482
24	Wreck	wreck nr. 70500	Dogger Bank, North Sea	4	25	Yes	SCUBA	10/09/2014	55.0370	1.7027
25	Wreck	wreck nr. 70501	Dogger Bank, North Sea	8	32.8	Yes	SCUBA	11/09/2014	54.8108	1.6898
26	Wreck	Britta	Cleaver Bank, North Sea		38.5	No	SCUBA	12/09/2014	53.9488	3.1618
16	Wreck	Russian submarine	Broadfourteens, North Sea		33	No	SCUBA	12/09/2014	53.0718	3.2326
27	Wreck	wreck “vaderdag”	Broadfourteens, North Sea	10	31	Yes	SCUBA	13/09/2014	52.4713	3.7827
28	Marina	Oost-Vlieland	western Wadden Sea	10	1	Yes	SCUBA	26/09/2014	53.2969	5.0890
15	Harbor	t Horntje	western Wadden Sea	8	3	Yes	Float	26/09/2014	53.0056	4.7964
1	Marina	West-Terschelling	western Wadden Sea	8	1	Yes	Float	26/09/2014	53.3651	5.2216
29	Marina	Kristineberg	Gullmar Fjord Skagerrak	17	1	Yes	Snorkel	30/09/2013	58.2499	11.4465

Checking epifauna for the presence of polyps and subsequent scraping epifauna from the bottom of floats was done in nine marinas at a minimum of three locations per site between March 2013 and September 2014. On site, the epifauna was inspected while submerged for the presence of polyps, which if present were collected the same way as described for the settling plates. Three polyps collected in 2010 in the marina of West-Terschelling by Floris Bennema were also included in the analysis. This location was re-sampled in September 2014. Additional polyps were collected from the underside of the dock of the Lovén Centre in Kristineberg, located at the Gullmar Fjord in western Sweden during a visit in September 2013.

Polyps were collected by recreational scuba divers at one site in lake Grevelingen and two sites in the western Wadden Sea. Additionally, polyps were collected by scuba diving on North Sea wrecks during two expeditions organized by Stichting Duik de Noordzee Schoon and the World Wildlife Fund on the vessel Cdt. Fourcault from 28 June–6 July 2013 and 5–13 September 2014. These expeditions aimed at investigating the biodiversity of North Sea shipwrecks and have been organised since 2011. Applied methods and results are published in Schrieken *et al.* (2013), Lengkeek *et al.* (2013b) and Coolen *et al.* (2015a). Dives were made by following a 45 m long reel line laid out by the first dive pair. Wreck parts in close (max 5 m) proximity to the line were inspected for the presence of scyphozoan polyps. The wrecks were not entered. When polyps were found, they were collected by scraping the polyps and their substrate using a filling knife with one hand, collecting the scrapings in a 1l Kautex jar held below the patch with the other hand. In hard-to-reach areas where only one hand could reach the polyp patch, polyps were collected by scraping slowly over the patch with the edge of a 50 ml centrifuge tube. Polyps were collected from as many different patches as possible. On board the polyps were cleaned and stored as described before. A minimum of 5 polyps per patch were collected.

DNA extraction and amplification

DNA was extracted from medusae and two to five (depending on availability) individual polyps per patch using the Power Soil DNA Isolation Kit (MO BIO Laboratories Inc.) following the manufacturers' protocol. DNA was eluted from silica columns in 50 µl of buffer, quantified with NanoDrop and run on 1 % agarose gels to verify the quality of the extract. Species identification was performed in two steps. For a first identification, diagnostic fragments of 650 bp of the V4 and V5 regions in the 18S rDNA gene were amplified from 2 µl of DNA extract in a 50 µl PCR using the primers EUK_F_566 and EUK_R_1200 according to Hadziavdic *et al.* (2014). The reaction mix contained 1× buffer, 200 µM dNTPs, 0,5 µM forward and reverse primers, 0.8 mg/ml BSA and 1 u Biotherm Plus Polymerase. PCR amplification started with 2 min at 95 °C followed by 35 cycles of 45 s at 95 °C, 60 s at 60 °C, 60 s at 72 °C and a final step of 7 min at 72 °C. Positive PCR controls (non-scyphozoan metazoa) and negative PCR controls were processed along.

To test whether the extraction- and PCR protocols worked correctly on scyphozoan polyps, the method was tested using three polyps of *Cyanea lamarckii* and three polyps of *C. capillata* provided from the cultures of Senckenberg am Meer by

Dr. Sabine Holst. PCR products were sequenced by BaseClear (Leiden) in a single run with forward primer EUK_F_566.

Subsequently, based on the genus to which the polyps were assigned, a fragment of fast evolving mitochondrial COI was amplified using a primer pair suitable for that genus, as different authors have used different primer pairs to amplify mitochondrial COI in the genera considered (Ramšak *et al.*, 2012; Lee *et al.*, 2013; Holst and Laakmann, 2014).

We used newly designed primers for analyses of intra-specific variation of *Aurelia aurita* polyps, based on GenBank data for the mitochondrial cytochrome c oxidase subunit I gene (COI) of *Aurelia aurita*: JQ623914, KC311384, KC311385, AY428838, AY903093–AY903095, AY903117, AY903208–AY903212, EF010537, DQ904436–DQ904439, FJ858784, EF010537, AY902911, AY902924; *Cyanea capillata*: AY902911, AY902924. *Rhizostoma pulmo* (as *R. octopus* was not available at the time): HQ902114–HQ902122, HQ904432–HQ904435, HQ999568–GQ999571:
Forward: ScyCOIf (5'-CTATACTTAATATTTGGTGCYTTTTC-3')
Reverse: ScyCOIr (5'-AAATGTTGGAATARTATTGGRTCTCCT-3')

PCR amplification started with 5 min at 94 °C followed by 40 cycles of 30 s at 94 °C, 45 s at 55 °C, 45 s at 72 °C and a final step of 7 min at 72 °C. Subsamples (5 µl) of all PCR products were loaded on 2 % agarose gels along with a size marker (SmartLadder SF) and stained with EtBr. The presence of bands was scored visually. Remaining volume (45 µl) of respective PCR products was submitted to BaseClear (Leiden) for purification and sequencing in two runs with primers ScyCOIf en ScyCOIr for intra-specific analyses of *Aurelia aurita*.

Data analysis

For species identification of the polyps, reads of the 18S rRNA gene were trimmed to 564 bp high quality fragments and, together with sequences from relevant Scyphozoa from Genbank, aligned to the Silva 119 reference database (Quast *et al.*, 2013) using ARB (Ludwig *et al.*, 2004). These sequences were added to the Silva guide tree using ARB Parsimony with positional variability settings specific for eukaryotes. Subsequently, from a subset of Scyphozoa sequences (1564–1684 bp) a small Maximum Likelyhood (ARB-RaxML) tree was built to which the sequences from this study (563-574 bp) were added using ARB Parsimony. Polyp species were identified based on the position of their sequences in the trees. For all polyps that were identified as belonging to the genus *Aurelia* based on their 18S rRNA sequence, forward and reverse sequences of COI were assembled and the consensus sequences were trimmed to 473 bp fragments. These were imported into Arlequin (Excoffier and Lischer, 2010) for standard diversity analyses, calculating pairwise F_{st} and analysis of molecular variance. A haplotype network was computed in R using the R package Pegas (Paradis, 2010). The genus *Aurelia* contains several cryptic species (Dawson *et al.*, 2005) and GenBank sequences of each species published in Dawson *et al.* (2005) were added to a COI tree to identify the polyps to species level.

Jellyfish polyps can reproduce asexually in different ways which means that polyps collected close to each other, for example in one patch, are likely to be clones. Including all polyps collected from a patch in an analysis of molecular variance would thus violate the assumption that individuals are sampled randomly from a population. For this reason, one polyp sequence from each polyp patch was randomly selected to be included in the analysis. patches were treated as separate if they were on a different SETL plate, a different host organism, or clearly separated by distance, for example on another wreck part. Sequences of this study are available from Genbank (KT962253-KT962259 for 18SrDNA and KP728285-KP728377 for COI)

Results

Polyp distribution

Jellyfish polyps were found inshore at four out of seven SETL settling plate locations and at seven out of nine locations were marine floats were sampled (Fig. 3.1b,c). In the Dutch and Great British Exclusive Economic Zone (EEZ) polyps were found at eight out of eleven dive locations; five locations on Dogger Bank and three in the "Broad Fourteens" area (Fig. 3.1a). Five of the wrecks were polyps were found were steamers, one a submarine, one a sunken oil rig (Ocean Prince) and one, the most recent wreck, the merchant motor vessel Vinca Gorthon sunk in 1988. Depths of these wrecks ranged from 20 m to 34 m. On the wrecks the polyps were generally found on parts that were sheltered from the current such as beam joints and the insides of pipes and boiler parts.

Polyps were found on a wide range of abiotic and biotic substrates (Table 3.2). Mostly, polyps were attached to the PVC of settling plates and to the oxidized metal surface of the wrecks. Polyps were also found on wood, granite pebbles, glass and synthetic rubber. A wide range of organisms was host to polyp patches; barnacles (both empty and alive), three species of bivalves, four species of tunicates, a sponge and a bryozoan.

Molecular identification

Nuclear 18S rDNA sequences of medusae and polyps used as reference material grouped with sequences of their respective genera in GenBank (Figure 3.3). The fragment analysed (563-574 bp) did not allow discrimination between *Rhizostoma pulmo* and *Rhizostoma octopus* and e.g. *Cyanea capillata* and *Cyanea annaskala*. 18S rDNA sequencing of polyps had a success rate of 85 % and worked for 183 samples. All sequences obtained from polyps were identical and matched to sequences of *Aurelia* sp. available in GenBank (Figure 3.3). Polyps of other genera were not detected. Subsequent analyses of the cytochrome-c-oxidase gene using the newly designed ScyCO primers was succesfull for 93 % of the Aurelia polyps. All polyps belonged to the species *Aurelia aurita* (Figure 3.5). Variation within

the 473 bp mitochondrial COI fragment was high with 52 variable sites and 50 different haplotypes with pairwise K80 distances ranging from 0–3 %.

Genetic structure

After randomly sampling one sequence per polyp patch to avoid including clonal genotype, 63 sequences remained which were used in further analyses (Table 3.4). Haplotype richness was high (h = 0.967 ± 0.011 on average) but differentiation among haplotypes was modest (π = 0.01080 ± 0.0007). Pairwise F_{ST} values indicated significant population differentiation between the Dogger Bank sample and other samples: Broad Fourteens, Skagerrak, Zeeland and Wadden Sea (Table 3.3); after Bonferroni correction, only the contrasts Dogger Bank versus Broad Fourteens, Wadden Sea and Zeeland remained significant. The largest level of genetic differentiation was found between the Broad Fourteens and Dogger Bank areas (F_{ST} = 0.489) and the smallest difference between the Wadden Sea and Zeeland (F_{ST} = -0.013). Based on the pairwise F_{ST} outcomes, two analyses of molecular variance (AMOVA) were carried out, both with sequence divergence taken into account. The first consisted of sites nested within two areas (outer area with Dogger Bank only versus coastal area with the other four samples). It showed a significant difference between outer and coastal areas but not among sites within areas (10.000 permutations, F_{ST} = 0.157 (p = 0.01), F_{SC} = -0.006 (p = 0.271), F_{CT} = 0.162 (p = 0.001). The second AMOVA was single-level with Dogger Bank versus the other samples pooled, confirming the different status of the Dogger Bank sample (10.000 permutations, F_{ST} = 0.227, p = 0.009).

A haplotype network was computed for the 473 bp mitochondrial COI fragments of all 183 *Aurelia aurita* polyps successfully sequenced in this study (Fig. 3.4) and showed that the most frequently found haplotype 1 was shared among all locations. The coastal areas Wadden Sea and Zeeland shared the most haplotypes with each other. The Dogger Bank area shared the least haplotypes with other areas.

One polyp COI sequence of each haplotype:location combination was deposited in GenBank (Table 3.5).

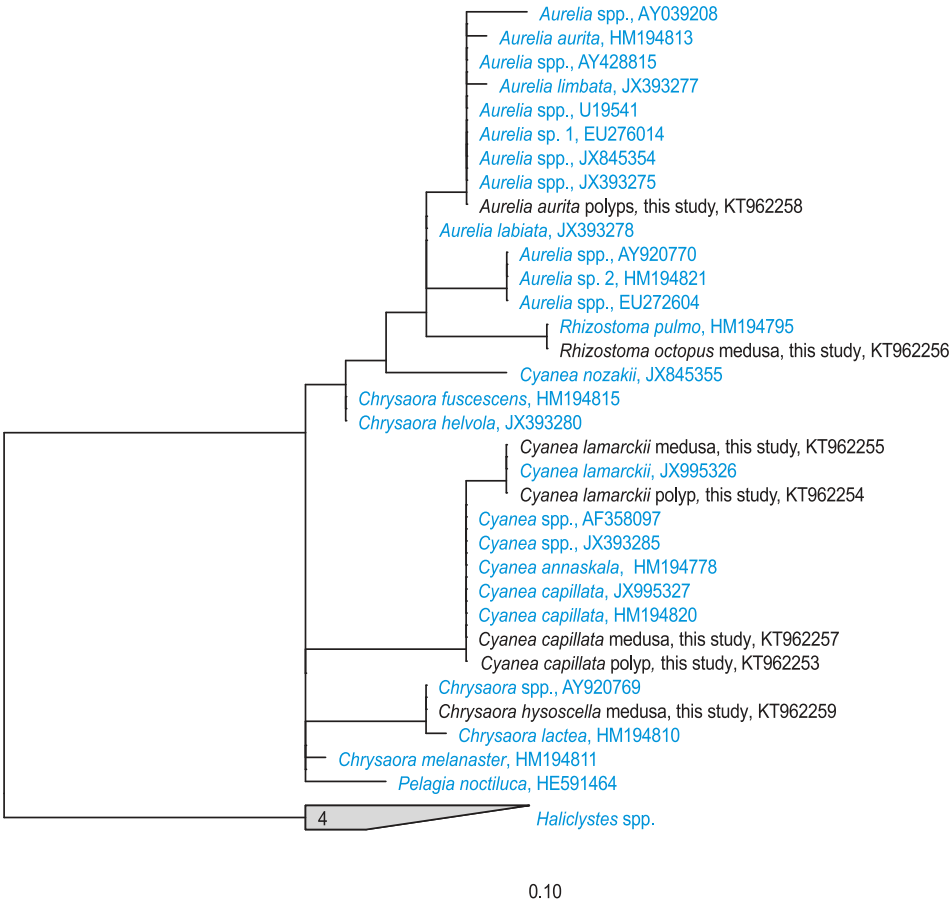

Figure 3.3: Maximum Likelyhood (ARB-RaxML) tree of partial 18S rRNA genes of Scyphozoa with sequences from this study in black (563-574 bp) added using ARB Parsimony (Ludwig *et al.*, 2004).

Table 3.2: Overview of substrates on which polyps were found with site numbers corresponding to the site number in Table 3.1

	substrate	site nr
abiotic	PVC	2,4,5,6
	Synthetic rubber	9
	Iron oxide (rust)	16,17,23,24,25,27
	Wood	29
	Granite	29
	Glass	29
biotic	*Crassostrea gigas*	7,15,28
	Mytilus edulis	1,9,12,15,28,29
	Pododesmus squamulatus	27
	Semibalanus/balanus sp.	1,13,15,28
	Encr. Bryozoan	27
	Aplidium glabrum	1
	Ascidiella aspersa	22
	Cyona intestinalis	7
	Styela clava	15,28
	Protosuberites sp.	1

Table 3.3: Pairwise F_{ST} values among samples of *Aurelia aurita* polyps in the North Sea area; Bonferroni-corrected threshold value is p = 0.005.

	Broadfourteens	Dogger Bank	Skagerrak	Wadden Sea
Dogger Bank	0.489***	-	-	-
Skagerrak	0.095	0.306**	-	-
Wadden Sea	0.050	0.310***	0.121	-
Zeeland	0.037	0.341***	0.096	-0.013

* p <0.05, ** p <0.005, *** p <0.001

Table 3.4: Sample sizes and standard diversity indices for partial COI sequences of *A. aurita* polyps sampled at five locations: N = sample size; N_h = number of haplotypes observed in sample; h = haplotype diversity; π = nucleotide diversity.

Area	N	N_h	h $\pm$ sd	$\pi \pm$ sd
Broad Fourteens	4	3	0.833 $\pm$ 0.222	0.00247 $\pm$ 0.0023
Dogger Bank	10	5	0.822 $\pm$ 0.097	0.00583 $\pm$ 0.00381
Skagerrak	7	6	0.952 $\pm$ 0.096	0.00785 $\pm$ 0.00516
Wadden Sea	18	14	0.967 $\pm$ 0.030	0.01270 $\pm$ 0.00710
Zeeland	29	18	0.936 $\pm$ 0.034	0.00976 $\pm$ 0.00549
Total	63	38	0.967 $\pm$ 0.011	0.01080 $\pm$ 0.0007

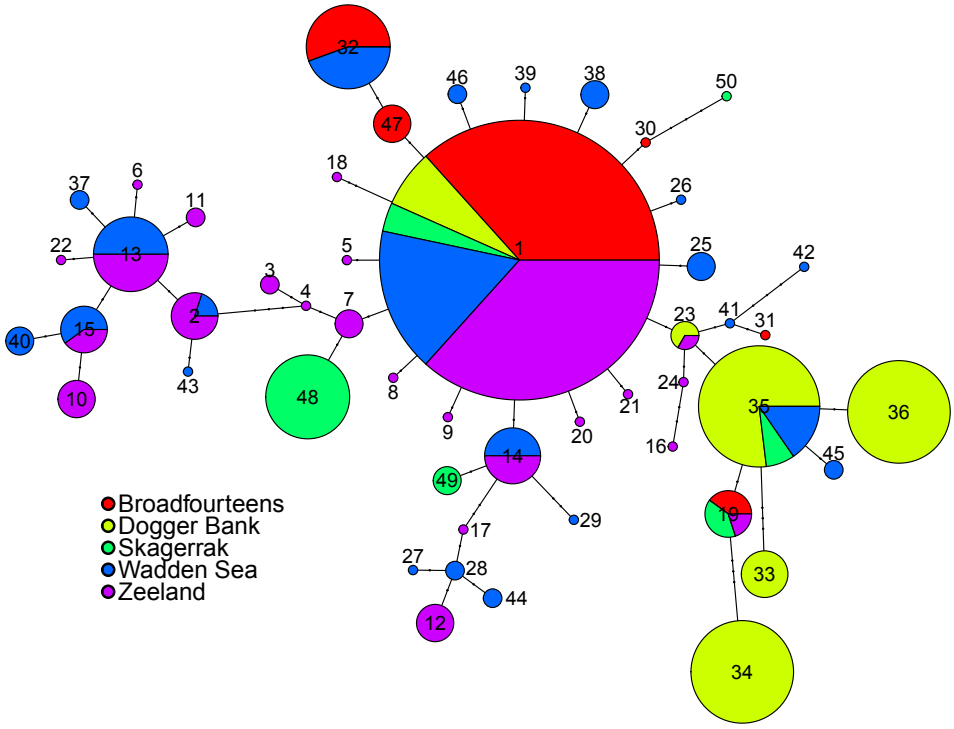

Figure 3.4: Haplotype network of the 473 bp mitochondrial COI fragments of all Aurelia aurita polyps sequenced in this study, computed using the R package “pegas”. Circle size is proportional to the frequency of the haplotype and circles are colored based on the proportion of individuals from the five different geographical areas sampled in this study. Lines represent mutational steps with black dots as hypothetical intermediate haplotypes.

Discussion

Aurelia aurita polyps were found in near-shore waters on settling plates, marina floats and other artificial as well as biological substrates, and offshore on several shipwrecks and an oil rig wreck on the Dogger Bank and the Broad Fourteens. *Aurelia aurita* polyps were found in most of the marinas and ports sampled in this study, suggesting marinas are an important source of *A. aurita* ephyrae in The Netherlands. Several of the Dogger Bank locations at which polyps were found, are in the Exclusive Economic Zone of the United Kingdom. In this area a total of nine gigawatt of offshore wind turbines is planned (Toke, 2011). Our study shows that settlement of scyphozoan planulae occurs in the Dogger Bank area. The structures installed for the wind farm would mean an increase in available polyp habitat and likely an increased release of ephyrae from the area, as has been hypothesized for wind farms in the Baltic Sea (Janßen *et al.*, 2013) as well.

Before the onset of industrial fisheries in the 1900s, large *Ostrea edulis* reefs were present on the Dogger Bank and in the area between the Dogger Bank and the Dutch-German coasts (Coolen *et al.*, 2015b). These reefs are now functionally extinct in the North Sea (Beck *et al.*, 2011), but may have provided a similar function to the bentic stages of jellyfish that is now provided by artificial structures.

Species composistion: only *Aurelia aurita*

There was sufficient variation in the 18S rDNA fragment sequenced to distinguish between the five species of metagenic scyphomedusae known to be present in the North Sea area but not enough variation to distinguish between all species at a worldwide scale (Figure 3.3). As several species of *Aurelia* are cryptic and are thought to be introduced species (Dawson *et al.*, 2005) it is possible that these also occur in the North Sea area unnoticed. Therefore, species identification using a faster evolving fragment, mtCOI, was required and applied.

Previous work mentioning the occurrence of jellyfish polyps in Dutch coastal waters assumed that these all belonged to *Aurelia aurita* (Leewis and Waardenburg, 1991; van Moorsel *et al.*, 1991; van Moorsel and Waardenburg, 1992a; van Moorsel, 1993; Lindeyer and Gittenberger, 2011; Gmelig Meyling *et al.*, 2013). In the present study all polyps were indeed assigned to *Aurelia* sp. based on 18S rDNA and *Aurelia aurita* based on COI. However, based on our data it cannot be excluded that in previous work polyps of other species were present and it is recommended that any future study on field-sampled polyps includes species identification based either on molecular identification or on the traditional method of rearing, strobilation and identification of ephyrae. Ephyrae of all North Sea species (Holst, 2012b,a) as well as many other species (Straehler-Pohl and Jarms, 2010; Straehler-Pohl *et al.*, 2011) can be identified to species level.

There are several possible explanations for the fact that only *Aurelia aurita* scyphistomae were found. Firstly it could be an effect of substrate preference. Experimental work by Holst *et al.* (2007) and Hoover and Purcell (2009) found that all species considered in this study settle on a variety of artificial substrates including plastic (PET). In the field, polyps of *Aurelia* species have been found

on artificial substrates in many areas worldwide (Duarte *et al.*, 2012). Polyps of *Cyanea capillata* were only found once in the field, on natural substrate; granite rock near the island of Helgoland in the German Bight (Hartlaub, 1894). In the Gullmar Fjord, despite the high abundance of *C. capillata* females bearing planula, few polyps colonized ceramic settling plates while the same plates were colonized extensively by *A. aurita* (Gröndahl, 1988) suggesting that there are differences in settlement preference.

Secondly, the different scyphozoan species could have preferences for different environmental conditions; if there are no adults in a certain area, no polyps are to be expected, either. Based on the more offshore distribution of *Cyanea lamarckii* and *C. capillata* in the North Sea, Hay et al. (1990) infer that the polyps of these species may prefer more saline and deeper waters. It was not anticipated that polyps collected in the Dogger Bank area of the central North Sea would all belong to *Aurelia aurita*, because *A. aurita* medusae are seldomly numerous in this area (Hay *et al.*, 1990). According to Thiel (Thiel, 1962) who studied *A. aurita* polyps in different locations in the Kiel Fjord, *A. aurita* polyps seem to prefer less saline waters.

Experimental observations on *Aurelia aurita*, *Cyanea lamarckii*, *C. capillata* and *Chrysaora hysoscella* indicate a high tolerance of their planulae and polyps to low salinity. Planulae of *C. capillata*, *C. lamarckii* and *C. hysoscella* settled in salinities down to 20 and polyps of *C. capillata*, *C. lamarckii* and *A. aurita* survived exposure to salinities down to 12 (Holst and Jarms, 2010) indicating that they can settle and survive in the estuaries bordering the southern North Sea including the Wadden Sea. Similar results were found for water temperature. For *Rhizostoma octopus* Holst *et al.* (2007) found that polyps could survive and reproduce at water temperatures experienced in the German Bight. In *A. aurita*, *C. lamarckii*, *C. capillata* and *C. hysoscella* strobilation activity differed between different temperature regimes but polyps of all these species survived and strobilated at average German Bight water temperatures as well (Holst, 2012a). Based on these observations, polyps of all species could potentially be found in the southern North Sea area.

The presence or absence of ephyrae in an area can be an indication whether polyps are present nearby. The presence of *Rhizostoma octopus* ephyrae in the Elbe estuary (Thiel, 1966) suggests that the polyps of this species would occur in less saline waters. Using similar reasoning, Merck (1989) suggests that the ephyrae of *Chrysaora hysoscella* might originate from the estuaries bordering the German Bight as they were found predominantly in the outflow of the river Elbe. In 2013, ephyrae of *C. hysoscella* and *Cyanea lamarckii* were found in plankton samples taken in the Eastern Scheldt and postephyrae of *Rhizostoma octopus* were found in the Wadden Sea (L. van Walraven unpublished data). Van der Baan (1980) studied the seasonal patterns of ephyrae and medusae of scyphomedusae extensively based on plankton samples taken in 1961-1966 from the lightvessel "Texel" 10 nm off the island of Texel. She found post-ephyrae of *Aurelia aurita* occasionally in January but most often in April and later. Small post-ephyrae of *C. hysoscella* were much less numerous and post-ephyrae of *R. octopus* were never observed, although Van der Baan (1980b) mentions that these were often observed in the Wadden Sea. Post-ephyrae (2-10 mm diameter) of *C. lamarckii* were found

from November to June in high densities.

Both medusa and polyp environmental preferences and tolerances have likely contributed to the lack of polyp species identifications other than *A. aurita* in the present study. The whereabouts of polyps of the other species in the southern North Sea region thus remains an open question.

Intra-specific variation in *Aurelia*

The separate genetic status of *Aurelia aurita* polyps on the Dogger bank confirms the observation by several other authors that populations of jellyfish may be differentiated at geographic scales of tens to hundreds of kilometre (see Dawson *et al.*, 2015b, for a review).

Variation in the COI gene of *Aurelia aurita* appears to be very high as 50 different haplotypes were found among the 183 polyps included in this study. A study that sampled medusae from southern English waters and the Irish Sea found 36 different haplotypes (Dawson *et al.*, 2015b), of which several were also found in this study. High levels of diversity are in fact to be expected in pelagic species with supposedly large population sizes, and this is indeed observed in many zooplankton taxa (see Peijnenburg and Goetze, 2013, for a review).

Analysis of the 473 bp COI sequences revealed population subdivision in the study area. The four nearshore and inshore areas Wadden Sea, Zeeland estuaries, Broad Fourteens and Skagerrak did not differ significantly from each other in contrast to the Central North Sea Dogger Bank area which differed from the Wadden Sea and Zeeland samples, and showed a trend towards differentiation from the Broad Fourteens and Skagerrak samples. The lack of connectivity may be a result ofthe prevailling hydrodynamic circulation and water currents. in the North Sea (Otto *et al.*, 1990). The Dogger Bank area mainly receives input of Atlantic water from the north, and not from the south (Turrell, 1992). Two studies modeling the dispersal of *Mnemiopsis leidyi* ctenophores from coastal areas to the North Sea found that particles released near the Dutch coast were rarely transported to the Dogger Bank area and vice versa (van der Molen *et al.*, 2015; David *et al.*, 2015). A similar pattern of transport could explain the genetic differentiation found between Dogger Bank and coastal *A. aurita* polyps.

Genetic diversity of *Aurelia aurita* polyps in the southern North Sea area was higher than that found for medusae of *Aurelia* sp. within Australia, California and Japan (Dawson *et al.*, 2005), within several European seas (Ramšak *et al.*, 2012) and between the Irish Sea and southern England (Dawson *et al.*, 2015b). Dawson *et al.* (2015b) show that genetically different populations of jellyfish of the same species can have different seasonal patterns and could respond differently to changing environmental conditions. Previous population genetic studies on scyphomedusae were based on medusae samples. The current study is the first that uses the sessile benthic stage and not the mobile free-living medusae, and shows that also in polyps population differentiation on similar spatial scales is found as for medusae. It would be interesting to investigate whether the phenotypes of these different populations are also different.

Several hurdles are encountered when sampling polyps rather than medusae.

The first is the problem of contamination, either by ingested prey material or by inclusion of the biotic substrate on which the polyps are living. Occasionally (11% of all processed polyps in our study) this resulted in complex electropherograms and mixed sequences when using universal primers such as EUK_F_566 and EUK_R_1200. The second aspect to take into account are the various modes of asexual reproduction used by the polyps, which means that multiple polyps sampled from the same patch are likely clones and can not be assumed to be independent. This reduces the amount of samples available for analysis of population structure. Using additional genetic markers such as microsatellite DNA would help to distinguish clones as well as provide higher resolution information on population connectivity (see e.g. Meirmans and Van Tienderen, 2004; Luttikhuizen *et al.*, 2007).

Polyps of the other species: where are they located?

Our study did not elucidate the location of the polyps of *Cyanea capillata*, *C. lamarckii*, *Chrysaora hysoscella* and *Rhizostoma octopus* in the southern North Sea area area although they should be present based on the occurrence of the medusae. Alternative methods could be employed to search for the origin of the medusae. When small medusae or ephyrae are found, hindcasting hydrodynamic models could be used to trace the medusae back to their origin, as shown by Dulière *et al.* (2014). Settling plates have been used to investigate settlement of planulae in an area (Gröndahl, 1988; Janßen *et al.*, 2013). Perhaps polyps of species other than *A. aurita* are more cryptic, i.e. not easily visible by eye, in which case bulk benthic scrapings from promising locations can be checked for the presence of polyps using next generation sequencing techniques or species specific primers and quantitative PCR as demonstrated in Bayha and Graham (2009), or micro-arrays (Ki *et al.*, 2010). Water samples can be checked for the presence of planulae of certain species, similar to what has been done for the identification of bivalve veligers (Philippart *et al.*, 2014). Environmental DNA sampling, which has been employed in the marine environment before (Thomsen *et al.*, 2012) could give useful information on the presence of nearby polyps when it is performed in periods when medusae are absent.

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Supplementary information

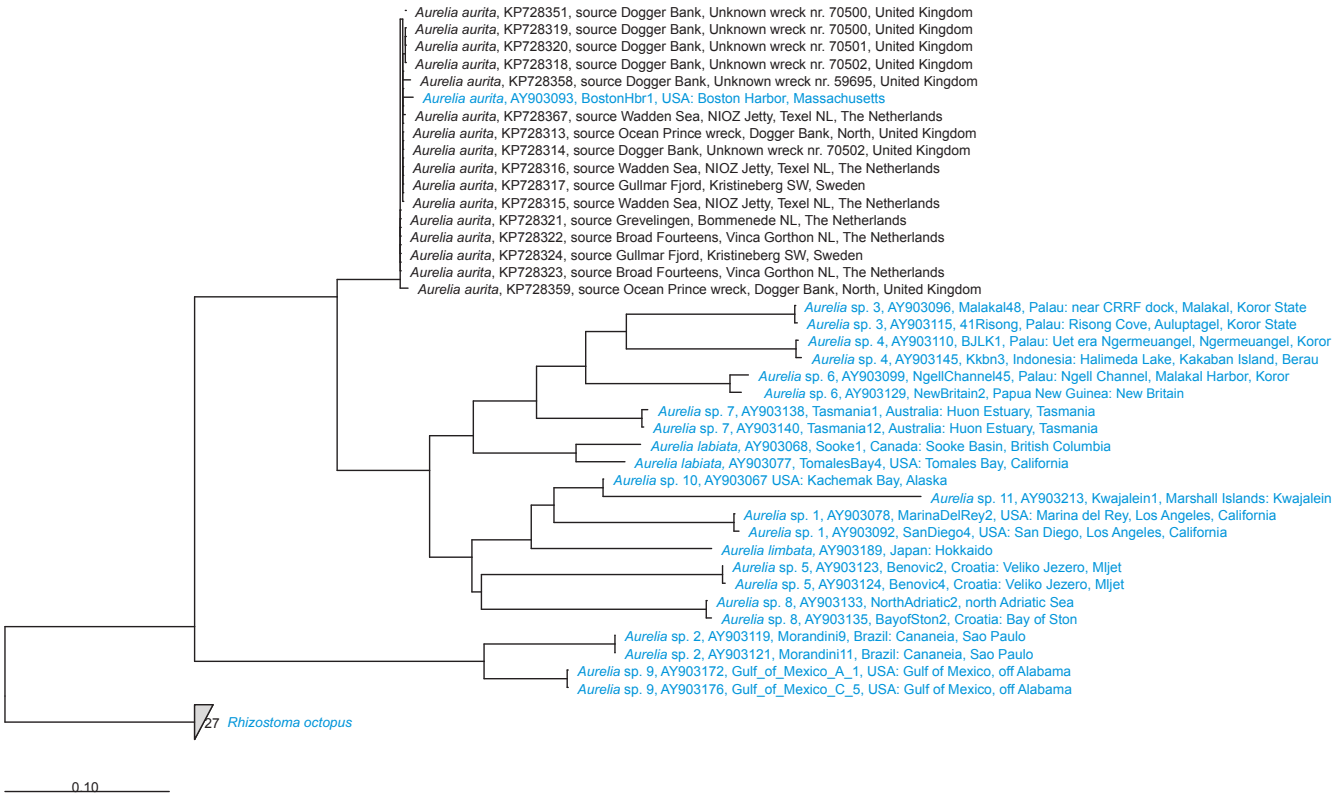
Table 3.5: List of COI sequences deposited in GenBank. pol: polyp, med: medusa, Aur: *Aurelia aurita*, Rhi: *Rhizostoma octopus*, haplo: haplotype number, corresponding with the numbers in fig. 3.4.*haplotype only found for medusa.

site	area	date	stage	species	haplo	accession nr
Marsdiep	Wadden Sea	23/04/2012	med	Aur	26	KP728373
Marsdiep	Wadden Sea	23/04/2012	med	Aur	51*	KP728374
Marsdiep	Wadden Sea	23/04/2012	med	Aur	1	KP728375
Vinca Gorthon	Broad Fourteens	05/09/2014	pol	Aur	1	KP728290
wreck "Vaderdag"	Broad Fourteens	13/09/2014	pol	Aur	1	KP728293
Ocean Prince	Dogger Bank	09/09/2014	pol	Aur	1	KP728313
wreck nr. 70500	Dogger Bank	10/09/2014	pol	Aur	1	KP728292
Kamperland	Zeeland	10/04/2013	pol	Aur	1	KP728285
Bommenede	Zeeland	18/03/2013	pol	Aur	1	KP728288
Den Osse Kerkweg	Zeeland	05/05/2013	pol	Aur	1	KP728286
Hompelvoet	Zeeland	18/03/2013	pol	Aur	1	KP728287
Kristineberg	Skagerrak	30/09/2013	pol	Aur	1	KP728298
Terschelling	Wadden Sea	26/09/2014	pol	Aur	1	KP728294
Terschelling	Wadden Sea	26/09/2014	pol	Aur	1	KP728295
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	1	KP728297
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	1	KP728296
Eemshaven	Wadden Sea	01/05/2013	pol	Aur	1	KP728289
Breskens	Zeeland	09/04/2013	pol	Aur	1	KP728284
Hompelvoet	Zeeland	18/03/2013	pol	Aur	2	KP728302
Den Helder	Wadden Sea	14/12/2012	pol	Aur	2	KP728303
Breskens	Zeeland	09/04/2013	pol	Aur	2	KP728301
Breskens	Zeeland	09/04/2013	pol	Aur	3	KP728332
Breskens	Zeeland	09/04/2013	pol	Aur	4	KP728333
Breskens	Zeeland	09/04/2013	pol	Aur	5	KP728334
Breskens	Zeeland	09/04/2013	pol	Aur	6	KP728335
Bommenede	Zeeland	18/03/2013	pol	Aur	7	KP728306
Hompelvoet	Zeeland	18/03/2013	pol	Aur	7	KP728305
Breskens	Zeeland	09/04/2013	pol	Aur	7	KP728304
Breskens	Zeeland	09/04/2013	pol	Aur	8	KP728307
Breskens	Zeeland	09/04/2013	pol	Aur	9	KP728336
Kamperland	Zeeland	10/04/2013	pol	Aur	10	KP728337
Kamperland	Zeeland	10/04/2013	pol	Aur	11	KP728338
Burghsluis	Zeeland	10/04/2013	pol	Aur	13	KP728328
Kamperland	Zeeland	10/04/2013	pol	Aur	13	KP728339
Hompelvoet	Zeeland	18/03/2013	pol	Aur	13	KP728329
Terschelling	Wadden Sea	26/09/2014	pol	Aur	13	KP728331
Den Helder	Wadden Sea	14/12/2012	pol	Aur	13	KP728330
Burghsluis	Zeeland	10/04/2013	pol	Aur	14	KP728340
Hompelvoet	Zeeland	18/03/2013	pol	Aur	14	KP728341
Eemshaven	Wadden Sea	01/05/2013	pol	Aur	14	KP728342
Bommenede	Zeeland	18/03/2013	pol	Aur	15	KP728309
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	15	KP728311
Den Osse Kerkweg	Wadden Sea	05/05/2013	pol	Aur	15	KP728308
Eemshaven	Wadden Sea	01/05/2013	pol	Aur	15	KP728310

site	area	date	stage	species	haplo	accession nr
Hompelvoet	Zeeland	18/03/2013	pol	Aur	16	KP728343
Hompelvoet	Zeeland	18/03/2013	pol	Aur	17	KP728344
Hompelvoet	Zeeland	18/03/2013	pol	Aur	18	KP728345
Vinca Gorthon	Broad Fourteens	05/09/2014	pol	Aur	19	KP728322
Vinca Gorthon	Broad Fourteens	05/09/2014	pol	Aur	19	KP728323
Bommenede	Zeeland	18/03/2013	pol	Aur	19	KP728321
Kristineberg	Skagerrak	30/09/2013	pol	Aur	19	KP728324
Bommenede	Zeeland	18/03/2013	pol	Aur	20	KP728346
Bommenede	Zeeland	18/03/2013	pol	Aur	21	KP728347
Bommenede	Zeeland	18/03/2013	pol	Aur	22	KP728348
wreck nr. 70502	Dogger Bank	10/09/2014	pol	Aur	23	KP728350
wreck nr. 70500	Dogger Bank	10/09/2014	pol	Aur	23	KP728351
Bommenede	Zeeland	18/03/2013	pol	Aur	23	KP728349
Bommenede	Zeeland	18/03/2013	pol	Aur	24	KP728352
Den Helder	Wadden Sea	14/12/2012	pol	Aur	25	KP728353
Den Helder	Wadden Sea	14/12/2012	pol	Aur	26	KP728354
Den Helder	Wadden Sea	14/12/2012	pol	Aur	27	KP728355
Terschelling	Wadden Sea	01/07/2010	pol	Aur	28	KP728312
Eemshaven	Wadden Sea	01/05/2013	pol	Aur	29	KP728356
Vinca Gorthon	Broad Fourteens	05/09/2014	pol	Aur	30	KP728300
Vinca Gorthon	Broad Fourteens	05/09/2014	pol	Aur	31	KP728357
Vinca Gorthon	Broad Fourteens	05/09/2014	pol	Aur	32	KP728325
Vinca Gorthon	Broad Fourteens	05/09/2014	pol	Aur	32	KP728326
Terschelling	Wadden Sea	26/09/2014	pol	Aur	32	KP728327
wreck nr. 59695	Dogger Bank	09/09/2014	pol	Aur	33	KP728358
Ocean Prince	Dogger Bank	09/09/2014	pol	Aur	34	KP728359
wreck nr. 70502	Dogger Bank	10/09/2014	pol	Aur	35	KP728314
Kristineberg	Skagerrak	30/09/2013	pol	Aur	35	KP728317
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	35	KP728315
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	35	KP728316
wreck nr. 70502	Dogger Bank	10/09/2014	pol	Aur	36	KP728318
wreck nr. 70500	Dogger Bank	10/09/2014	pol	Aur	36	KP728319
wreck nr. 70501	Dogger Bank	11/09/2014	pol	Aur	36	KP728320
Vlieland	Wadden Sea	26/09/2014	pol	Aur	37	KP728360
Vlieland	Wadden Sea	26/09/2014	pol	Aur	38	KP728361
Vlieland	Wadden Sea	26/09/2014	pol	Aur	39	KP728362
Vlieland	Wadden Sea	26/09/2014	pol	Aur	40	KP728363
Vlieland	Wadden Sea	26/09/2014	pol	Aur	41	KP728299
Terschelling	Wadden Sea	26/09/2014	pol	Aur	42	KP728364
Terschelling	Wadden Sea	26/09/2014	pol	Aur	43	KP728365
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	44	KP728366
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	45	KP728367
Texel (NIOZ)	Wadden Sea	26/09/2014	pol	Aur	46	KP728368
Russian Submarine	Broad Fourteens	03/07/2013	pol	Aur	47	KP728369
Kristineberg	Skagerrak	30/09/2013	pol	Aur	48	KP728370
Kristineberg	Skagerrak	30/09/2013	pol	Aur	49	KP728371
Kristineberg	Skagerrak	30/09/2013	pol	Aur	50	KP728372
wreck nr. 70502	Dogger Bank	10/09/2014	pol	Aur	1	KP728291
Marsdiep	Wadden Sea	23/07/2012	med	Rhi		KP728376
Marsdiep	Wadden Sea	23/07/2012	med	Rhi		KP728377

Figure 3.5: Neighbour Joining tree of COI sequences of *Aurelia aurita* with sequences from this study in blue, with pairwise distances estimated using the F81 model (Felsenstein, 1981). *Rhizostoma octopus* is used as outgroup.

Figure 3.5: (cont.) Neighbour Joining tree of COI sequences of *Aurelia aurita* with sequences from this study in blue, with pairwise distances estimated using the F81 model (Felsenstein, 1981). *Rhizostoma octopus* is used as outgroup.



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Chapter 4

Seasonal occurrence of the invasive ctenophore *Mnemiopsis leidyi* in the western Dutch Wadden Sea

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Abstract

The ctenophore *Mnemiopsis leidyi* is an invasive species in northern European waters since 2006. This paper presents the first quantitative data for the western Dutch Wadden Sea based on weekly measurements year-round in 2009 of abundance and size distribution. Due to the short residence time of the water, the seasonal occurrence of this species in the western Dutch Wadden Sea is a reflection of its occurrence in the Dutch coastal zone of the North Sea. *M. leidyi* was present the whole year round with multiple peaks. Spawning started in May and was followed by an increase in density of 3 orders of magnitude, leading to the first peak in mid-June (highest mean density 360 ind m^{-3}). After a decrease in July numbers increased again and a second peak occurred in mid-August (highest mean density 342 ind m^{-3}) during which the highest density of 912 ind m^{-3} in a single haul was observed. At peak densities the population consisted almost entirely of small (< 20 mm) ctenophores. Density and biomass remained high until October, decreasing to low levels in November. Predation by *Beroe gracilis* was observed, however the low densities suggested only a minor impact on *M. leidyi*. Predatory impact of *M. leidyi* on fish larvae in the Wadden Sea is likely to be restricted because most fish species spawn early in the year before the first peak of *M. leidyi*. Nevertheless, through competition for food with other zooplanktivores, the species could have a major influence on the Wadden Sea ecosystem.

Introduction

Ctenophores are considered to be important predators and hence components of planktonic ecosystems (Mills, 1995). There are indications that gelatinous zooplankton blooms are increasing in magnitude and frequency (Gibbons and Richardson, 2009) as a consequence of new invasions and a range of anthropogenic factors such habitat modification, eutrophication, climate change and overfishing (Mills, 2001). During the last decades one such invasion in the coastal waters of north-western Europe has been by the sea walnut *Mnemiopsis leidyi* A. Agassiz, 1865 (Costello *et al.*, 2012).

M. leidyi is an opportunistic planktonic predator, feeding on a wide range of different zooplankton prey such as copepods and their nauplii, bivalve veligers, barnacle nauplii (Granhag *et al.*, 2011; Javidpour *et al.*, 2009a), fish larvae (Cowan and Houde, 1992) and eggs (Purcell *et al.*, 1994). It is a simultaneous hermaphrodite, and this feature along with its high fecundity and very high growth rates allow it to increase very rapidly in numbers and biomass under favorable conditions (Purcell *et al.*, 2001) and to form large blooms, which can exert a large predation pressure on zooplankton communities and thus cause a decrease in food availability for other zooplanktivorous animals.

In the 1980s the first invasion of *M. leidyi* to waters outside its native range was in the Black Sea, most likely via ballast water of oil tankers. After its first sighting in 1982 the *M. leidyi* population remained at relatively low levels until 1989, when its density and biomass exploded following recruitment failure in the dominant zooplanktivorous fish species, the anchovy Engraulis encrasicolus (Bilio and Niermann, 2004) due to overfishing. This lack of competition combined with climate induced enhanced carrying capacity led to a competitive advantage of *M. leidyi* over pelagic fish, which contributed to further anchovy stock depletion (Oguz *et al.*, 2008). The collapse of pelagic fish stocks had a large impact on fisheries in the region (Knowler, 2005). A few years later, the natural predator of *M. leidyi Beroe ovata*, was also introduced in the Black Sea and subsequently *M. leidyi* blooms decreased in frequency and magnitude (Shiganova *et al.*, 2001). In recent years, *M. leidyi* blooms have been observed outside the Black Sea along the Mediterranean Sea coasts of Spain and Israel (Fuentes *et al.*, 2010).

In north-western Europe, the number of reports of *M. leidyi* increased when molecular identification confirmed the presence of the species (Faasse and Bayha, 2006). By then it had been sighted in coastal waters off Sweden (Hansson and Kiørboe, 2006), Germany (Javidpour *et al.*, 2006) and The Netherlands (Faasse and Bayha, 2006; Tulp, 2006). Its presence in North-western European coastal waters has now also been reported from Denmark (Tendal *et al.*, 2007), Germany in the North Sea (Boersma *et al.*, 2007) as well as in the Baltic Sea (Javidpour *et al.*, 2006), Poland (Janas and Zgrundo, 2007) and Norway (Oliveira, 2007).

M. leidyi has most likely been present earlier in Northern Europe, in the past mistakenly identified as *Bolinopsis infundibulum* O.F. Müller, 1776. In Dutch waters for example, *M. leidyi* was probably present as early as 2002 (Holsteijn, 2002) and maybe even from 1992 in Lake Grevelingen (Faasse and Ligthart, 2007). Despite its presence and the possible influence it may have on ecosystems and

especially on zooplanktivorous fish stocks (Purcell *et al.*, 2001), no investigations were carried out so far on *M. leidyi* in the Dutch Wadden Sea which is an important nursery ground for various fish species (Zijlstra, 1972).

Previous studies on gelatinous zooplankton in Dutch coastal waters were only carried out in the 1980s (Van der Veer and Sadée, 1984; Van der Veer, 1985; Miller and Daan, 1989; Kuipers *et al.*, 1990). Therefore, a year-round quantitative sampling programme was set up in 2009. This paper presents information on the seasonal occurrence of *M. leidyi* in the western Dutch Wadden Sea and discusses the factors influencing its population dynamics in this estuarine area and its potential role as predator on the zooplankton community, including fish and bivalve larvae.

Material and methods

Field sampling

All samples were taken in the Marsdiep and Vlie basin in the western Dutch Wadden Sea in various 5–15 m deep tidal gullies during January to December 2009 (Fig. 4.1). In principle, two or more ebb and flood tides were sampled weekly during daytime and during each tide about 5 hauls were made from anchored vessels. Nets and flow meters were the same as used in previous studies on gelatinous zooplankton in the area (Van der Veer and Sadée, 1984; Van der Veer, 1985).

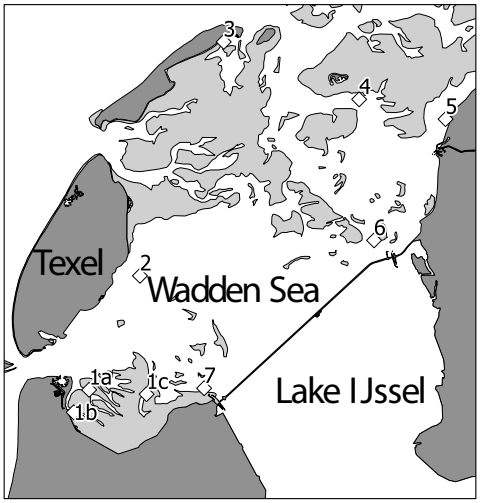

Figure 4.1: Sampling stations in the western Wadden Sea. Stations 1a,b,c are Balgzand stations (c.f. Van der Veer and Sadée, 1984). The other stations are Texelstroom (2), Kornwerderzand (6) and Den Oever (7) in the Marsdiep basin and Vlieland (3), Blauwe Slenk (4), Kimstergat (5) in the Vlie basin.

Oblique hauls were made with nets made of polyamide plankton gauze (Monodur ® 2000, 2 mm mesh size) with an opening of 0.7 m^{-2}, a length of 5 m, a porosity of 0.59 and a total surface area of 12 m^{-2}. The maximum depth, average

depth and duration of each haul were measured with a Suunto D3 dive computer attached to the net frame. The amount of water passing through the net was registered with a Savonius type flow-meter mounted in the mouth of the net. Porosity and mesh area of the net (definitions according to Smith *et al.* (1968)) were large enough to prevent any serious clogging and overflow even during the spring bloom of the algae *Phaeocystis* sp. (Van der Veer and Sadée, 1984).

The general reduction of water flow through the net was about 10% and independent of current velocity. Haul duration varied depending on current velocity, mesoplankton density and density of the alga *Phaeocystis* sp. Haul duration ranged between 3 min at high current velocities and 67 min at very low current velocities, and hence volume filtered per haul differed between 32 and 1099 m^{-3}. In addition, water temperature and salinity was measured at the surface once during the haul with a hand-held conductivity meter (accuracy $\pm$ 0.1 unit).

Depending on volume, the catch was placed directly in a 1 mm sieve after net wash down, or in the case of >5 l emptied in a 60 l bucket and then washed down above the sieve. Small samples were sorted freshly on board, otherwise the sample or a subsample was fixed and preserved using the slightly modified method of Adams *et al.* (1976) (see Appendix). For this, the sample or a subsample of maximum 500 ml was put in an 800 ml glass jar, which was then filled up with the 10 g/l trichloroacetic acid (TCA)-seawater fixation solution. Subsequently, the samples were transported to the lab. In summer, the fixed samples were stored in a Styrofoam box with ice for cooling. In the laboratory the TCA-fixed samples were transferred on the day they were caught to a 1% solution of 10 ml preservation stock solution of 5 ml propylene phenoxetol, 45 ml propylene glycol and 50 ml 40% formaldehyde and 990 ml seawater. For this, samples were drained in a sieve of ca. 0.5 mm mesh size, returned in the jar and subsequently the preservation solution was added. After 5–8 days the samples were transferred to a 5% solution of 50 ml preservation stock solution and 950 ml seawater. Samples were stored at a constant temperature of 4 °C.

Laboratory measurements

Samples were sorted in black sorting trays and of each individual the distance from the mouth to the statocyst was determined. Ctenophores in length < 10 mm were measured submerged in a petri dish using a stereo microscope with a measuring eyepiece. Larger ctenophores were measured using a Vernier caliper. Subsampling of large densities of small ($\leq$ 5 mm length) ctenophores was done using a Folsom plankton splitter. Individuals > cm have been considered as adults Rapoza *et al.* (c.f. 2005). Measurements on preserved individuals were corrected for shrinkage and converted into fresh length and fresh wet mass (see Appendix).

Data analysis

M. leidyi densities were log transformed to stabilize the variance. Analysis of the raw data was not possible due to high levels of autocorrelation, therefore we

calculated weekly averages for density, temperature, and salinity and used this for further analyses.

Scatter plots of *M. leidyi* density versus time and versus the covariates showed non-linear patterns and therefore generalized additive models were applied (Wood, 2006). These models assume homogeneity, normality and independence of residuals. To verify these assumptions, residuals of the models were inspected for temporal correlation using the auto-correlation function. Normality and homogeneity of variance of the residuals was also verified using histograms and plots of residuals versus fitted values (Zuur *et al.*, 2009). All calculations were carried out in R version 2.14.1 (R Core Team, 2014). The GAM was applied with the gam function in the mgcv package (Wood, 2006).

To investigate whether the relationship of abiotic factors temperature and salinity differed between the start and end of the season, the year was split into two periods (per) (week 3–26 and week 28–49). Model selection was performed using stepwise backwards selection. In the full model, the base 10 logarithm of *M. leidyi* density *D* was estimated by a model with intercept α, different smoother f for temperature and salinity, which also differed per period and an error term ϵ:

$$log(D)_{per} = \alpha + f(temperature_{per}) + f(salinity_{per}) + \epsilon \quad (4.1)$$

The relationship between temperature, salinity and start of spawning was investigated by determining the presence or absence of *M. leidyi* juveniles of $\leq$5mm length (hereafter called 'larvae') for every sampling week. Only data from the first half of the year (week 3–26) was used because in the second half of the year this size class was present in all but one week. Logistic regression was used to model the probability of *M. leidyi* larvae presence *p* in relation to water temperature *t* and salinity *s*. These models assume that the data are distributed according to a binomial distribution and that residuals are homogenous and independent. Model selection was performed using stepwise backwards selection. *logit(p)* was estimated by a model with an intercept α, a parameter for temperature (β_t) and salinity (β_s) and error term ϵ:

$$logit(p) = \alpha + \beta_t * temperature + \beta_s * salinity + \epsilon \quad (4.2)$$

Data analysis was carried out using R (R Core Team, 2014) and SigmaPlot® 11.0.

Results

Abiotic factors

Seawater temperature ranged from 1.7 °C in February to maxima of 18–21 °C in Ma–August, followed by a decrease again to 4.1 °C in December (Fig. 4.2). Salinity was very variable over the sampled period and also between tidal phases. Salinity decreased from 30 in January to a minimum of 15 in April, after which it increased again to varying levels > 22, except for one week in June and December. Salinity was generally higher during flood than during ebb tide, except in late summer/autumn (Fig. 4.2).

Ctenophore data

M. leidyi was present the whole year round with lowest densities in February (Fig. 4.3 and 4.4). Catches were highly variable with fluctuations of orders of magnitude even within a single tide. Numbers remained low (at or below 1 ind m^{-3}) until mid-May. The first increase occurred in June, with a mean density of 360 ind m^{-3}. Hereafter, numbers decreased to a minimum of 0.7 ind m^{-3} at the end of July, followed by a second increase with a mean density of 342 ind m^{-3} at the end of August. A week later, there was a 20-fold decrease in density. The highest density in a single haul was 921 ind m^{-3} on August 18. In winter and spring the population consisted of a mixture of small individuals between 5 and 20 mm in length and ctenophores larger than 20 mm. In spring the percentage of larger ctenophores > 20 mm slowly increased until the first wave of juveniles < 5 mm length entered the catches in May. During the peak ctenophores < 5 mm dominated. After the spring peak the catches consisted mainly of ctenophores <20 mm, the percentage larger ctenophores exceeded 10% rarely (Fig. 4.4).

Mean biovolume remained below 1 ml m^{-3} in winter, until it increased in the second week of April (Fig. 4.5). In mid-May, wet mass peaked, coinciding with the increase in density. The trend in wet mass was the same as in density with two peaks, the first in mid-June (68.3 ml m^{-3}). Hereafter, wet mass decreased to 1.7 ml m^{-3} at the end of July. Next, a second increase occurred with 55.6 ml m^{-3} at the end of August. This peak lasted only one week, after which it decreased again. The highest observed wet mass in a single haul was 204 ml m^{-3} on October 29th.

A 3D plot of *M. leidyi* density versus water temperature and salinity (Fig. 4.6) showed that the highest densities are reached at the highest water temperatures and at salinities > 15. The smoothing function for salinity was not a significant factor in the GAM analysis of weekly averages of *M. leidyi* density in relation to abiotic factors, so the simplest model was:

$$log(D)_{per} = \alpha + f(temperature_{per}) + \epsilon \tag{4.3}$$

Water temperature was significant in both periods (Table 4.1). A plot of fitted values of the model using different smoothers for each period (Fig. 4.7) showed a clear positive relationship between water temperature and *M. leidyi* density in the first half of the year. The temperature effect increased at around 12 °C and levelled off at around 17 °C. The fitted model for the second half of the year showed a more complicated relationship between water temperature and *M. leidyi* density, where twice the temperature effect became negative and then positive again. At temperatures < 8 °C the confidence interval was very wide.

With respect to the start of spawning, all parameters were significant except salinity (Table 4.2) and the remaining model was:

$$logit(p) = \alpha + \beta_t * temperature + \epsilon \tag{4.4}$$

The probability of *M. leidyi* larval presence (ctenophores < 5 mm) increased with water temperate and was close to 100% at temperatures > 15 °C (Fig. 4.8).

A few other ctenophore species were found: *Pleurobrachia pileus* (O. F. Müller, 1776) numbers started to increase at the end of March, and a first peak occurred

Table 4.1: Model results of the Generalized Additive Model describing the relationship between *M. leidyi* density and water temperature. Each half of the year has a different smoother (period 1 and 2). For each smoothing term the estimated degrees of freedom are given along with the *F*-statistic and *p*-value.

	Estimate	Std. Error	*t* value	*p*-value
Intercept	0.54	0.09	5.8	<0.001
		estimated *df*	F	
s(temperature):period 1		4.56	25.4	<0.001
s(temperature):period 2		7.56	9.11	<0.001
R^2(adj) = 0.85 Deviance explained = 89.3% AIC = 68.15				

Table 4.2: Model results of the logistic regression model describing the relation between probability of *Mnemiopsis leidyi* larvae < 5 mm length presence and water temperature.

Estimate	Std. Error	*z* value	*p*-value	
intercept	-4.88	2.02	-2.41	0.016
temperature	0.53	0.20	2.65	0.008
Null deviance: 31.49 on 22 degrees of freedom				
Residual deviance: 12.40 on 21 degrees of freedom. AIC: 16.40				

in mid-May with a mean density 2.7 ind m^{-3}. Over the next two weeks the numbers decreased to a mean density of 0.3 ind m^{-3} in the beginning of June before increasing again to 3.5 ind m^{-3} at the end of June, which coincided with the first peak in *M. leidyi*. Hereafter, numbers decreased; after mid-July the species was absent with only a few individuals sampled at the end of August (Fig. 4.9). *Beroe gracilis* (Künne, 1939) was present only in summer with a mean peak density of 0.3 ind m^{-3} in mid-June, coinciding with the first peak density of *M. leidyi* and the second peak density of *P. pileus*. After the beginning of July it had almost disappeared. The individuals caught on June 16 were examined alive in the lab under a dissection microscope, and in one individual an ingested juvenile of *M. leidyi* was found.

Scyphozoans

The abundance of scyphozoans was remarkably low. *Aurelia aurita* L. 1758, *Chrysaora hysoscella* L. 1767 and *Cyanea lamarckii* Péron and Lesueur, 1810 were almost absent. *Rhizostoma octopus* L. 1758 was the most abundant scyphomedusa, present from mid-June until the beginning of September, with a maximum density of 0.02 ind m^{-3} in the end of August.

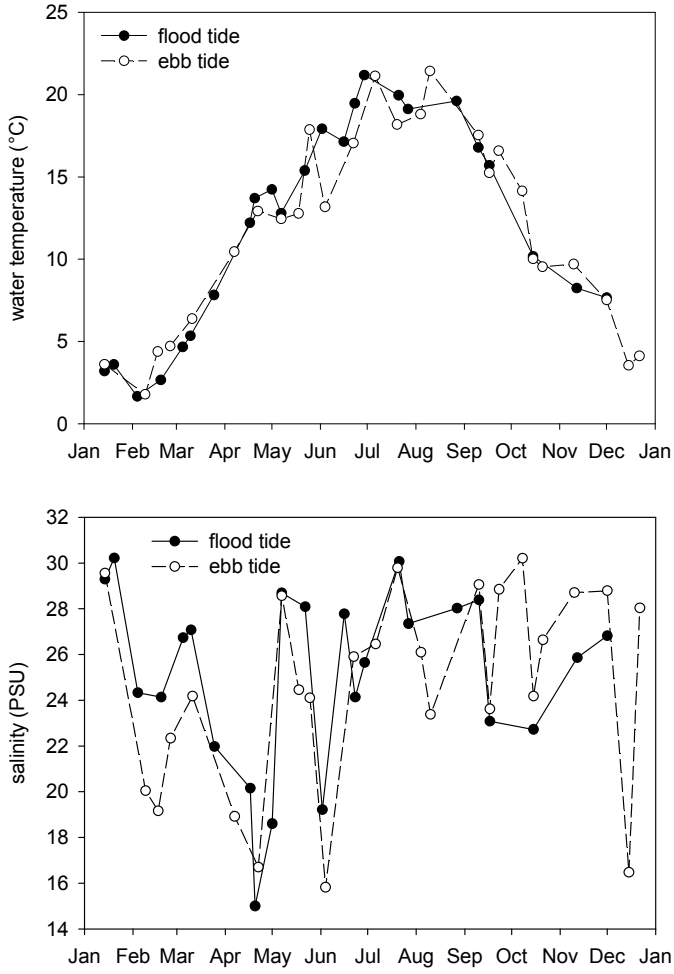

Figure 4.2: Mean surface water temperature (SST) and salinity (SSS) of all sampling stations per week during flood and ebb tides in 2009.

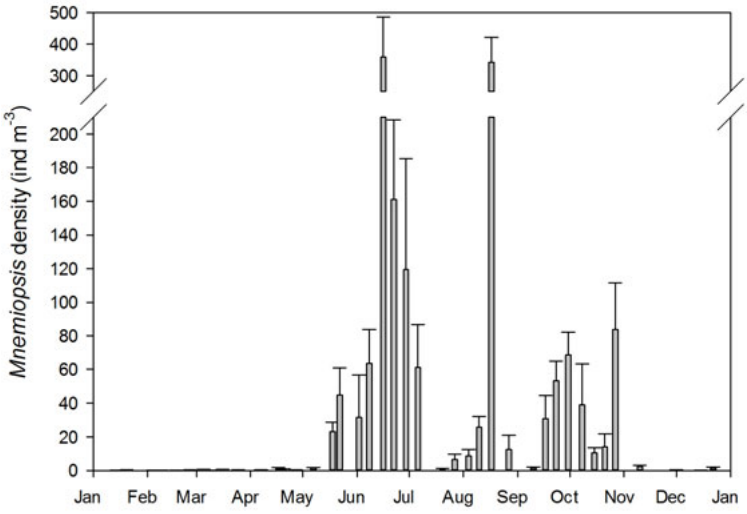

Figure 4.3: Mean density ± SE (ind m^{-3}) of *Mnemiopsis leidyi* in the western Wadden Sea in 2009. All stations and hauls together.

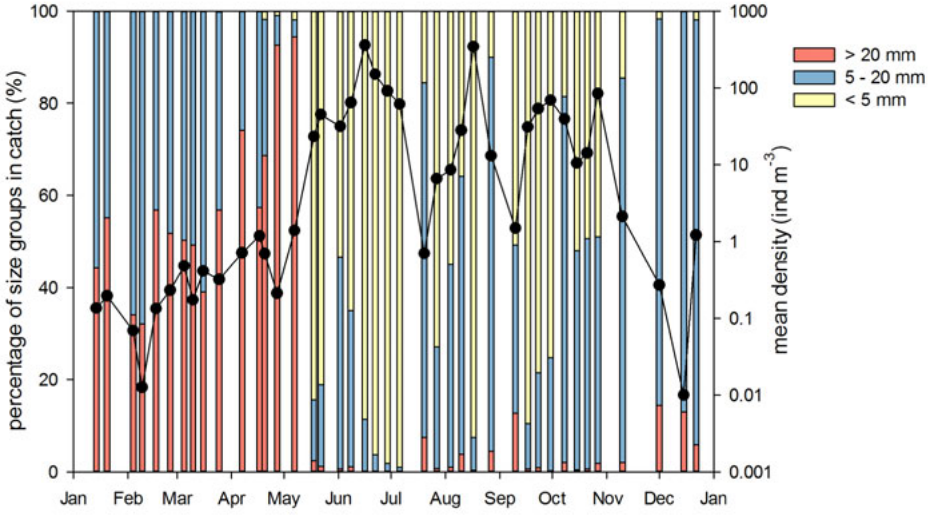

Figure 4.4: Mean length distribution of three different size groups (bars) of *Mnemiopsis leidyi* of all sampling stations in the western Wadden Sea in 2009, together with total density (black dots; right axis)

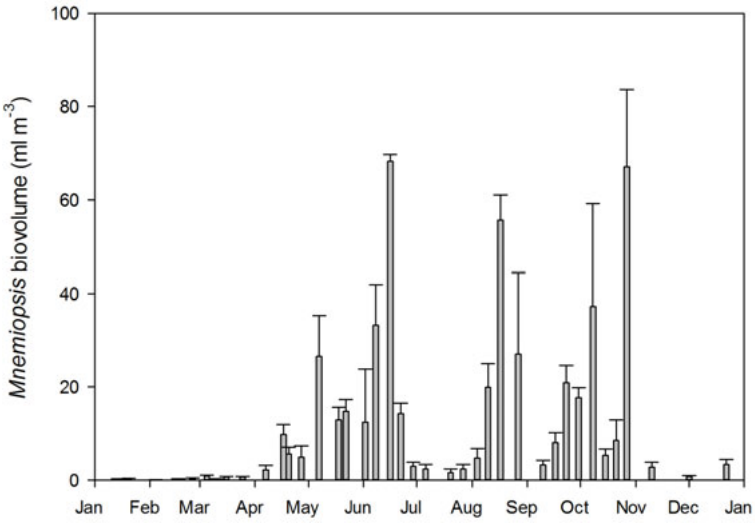

Figure 4.5: Mean biovolume of *Mnemiopsis leidyi* (wet mass; ml m^{-3}) in the western Wadden Sea in 2009. All stations and hauls together.

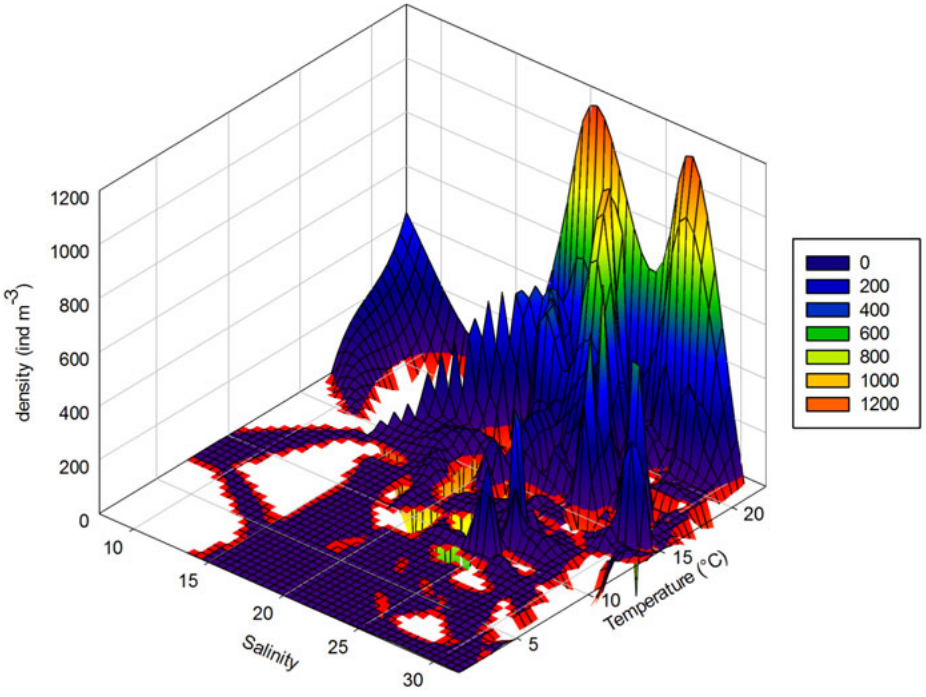

Figure 4.6: 3D graph of *Mnemiopsis leidyi* density ind m^{-3}) versus water temperature and salinity per haul for all stations in 2009.

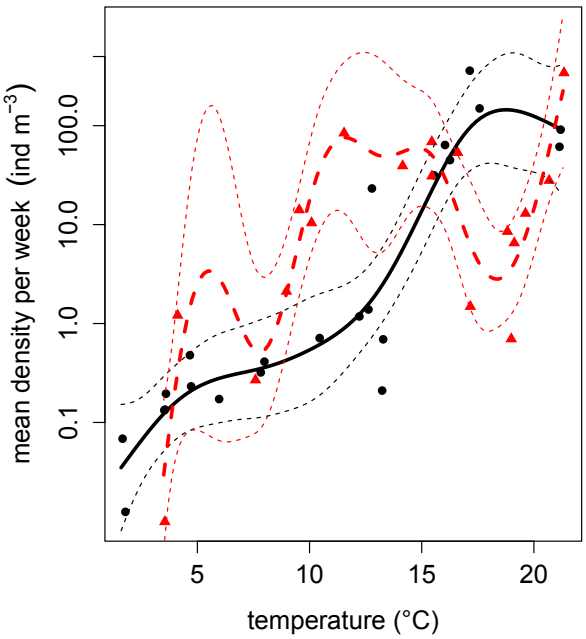

Figure 4.7: Mean *M. leidyi* density per week for all sampling stations in 2009 for the first half (●) and second half (▲) of the year, together with the fitted smoothing functions of the GAM model for the first half (solid, black) and second half (dashed, red) of the year and their confidence intervals.

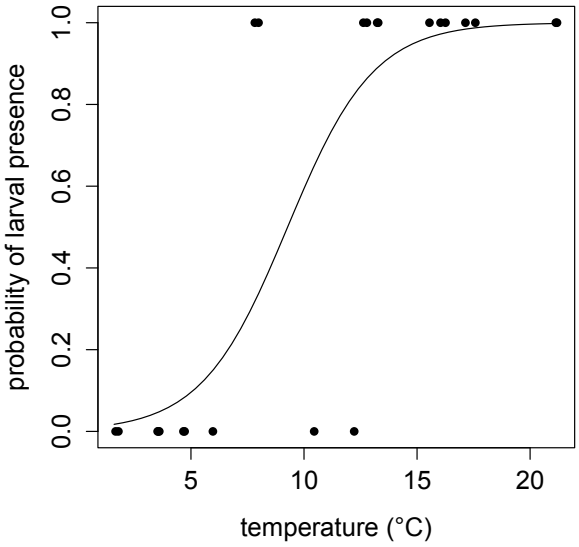

Figure 4.8: Presence and absence of *M. leidyi* individuals < 5 mm per week in relation to temperature, together with the fitted logistic regression model.

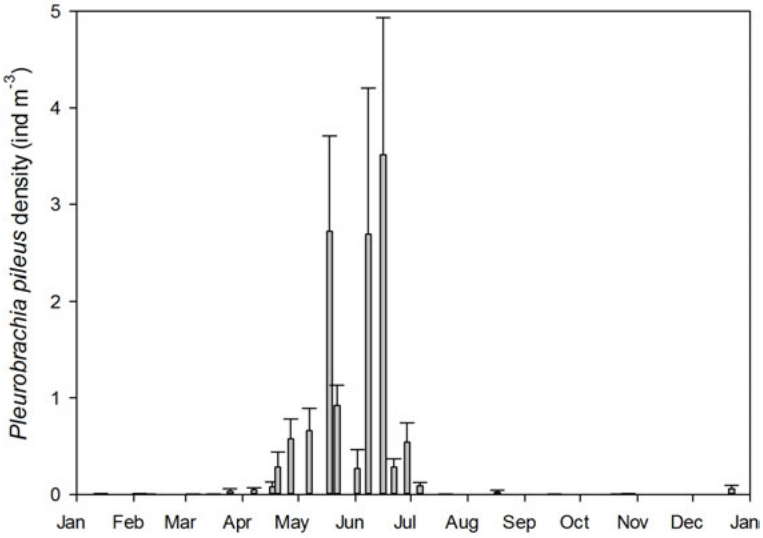

Figure 4.9: Mean weekly density and SE (ind m^{-3}) of *Pleurobrachia pileus* in the western Wadden Sea in 2009. All stations and hauls together.

Discussion

In 2006 *M. leidyi* was observed in Dutch coastal waters from August to December (Faasse and Bayha, 2006) but this study shows that in 2009 *M. leidyi* appeared to be present year-round at least in the western Dutch Wadden Sea. This area is relatively well mixed with only weak vertical salinity stratification (Postma, 1954; Zimmermann, 1976). Approximately 70% of the water in the western Wadden Sea is renewed every week by sea water from the North Sea and fresh water from Lake IJssel (Zimmermann, 1976).

Ridderinkhof *et al.* (1990) estimated the mean turnover time for the Marsdiep basin as 17 tidal cycles (ca. 8.5 days), and that of the Vlie tidal basin as 13 tidal cycles (6.5 days). Nevertheless, particles such as sediment can accumulate in these basins by a mechanism of being swirled up and transported more during the generally stronger flood tides than being washed back with the relatively weaker ebb flows van Straaten and Kuenen (1958); Postma (1961)(Van Straaten and Kuenen, 1958; Postma, 1961). This mechanism can also result in an import and accumulation of planktonic organisms, especially small ones such as barnacle (de Wolf, 1973) and flatfish larvae (Rijnsdorp *et al.*, 1985; Van der Veer, 1986). A similar mechanism might apply for ctenophores (de Wolf, 1989).

M. leidyi exhibited multiple peaks in the western Wadden Sea in contrast to other areas in the northern part of its native range, where it exhibits one annual peak in biomass, which generally occurs in late summer and autumn (Table 4.3). The more irregular and short nature of *M. leidyi* peaks in the western Wadden Sea may support the view that in the Wadden Sea it is a sink population (Costello *et al.*, 2012). In the Kiel Bight the *M. leidyi* population also exhibited the same seasonal patterns as observed in this study; multiple short peaks (Javidpour *et al.*, 2009a). The high variation in densities of *M. leidyi* in catches made even within a single tide suggests that even in the turbulent and well-mixed channels of the Wadden Sea *M. leidyi* has a very patchy distribution. This is in line with previous observations on a range of different zooplankton species in the Wadden Sea (de Wolf, 1989).

Table 4.3: Highest recorded mean density and bio-volume of *Mnemiopsis leidyi* in different areas.

Area	Highest means of:		month(s) of peak biomass	Reference
	density ind m^{-3}	biovolume ml m^{-3}		
Narragansett Bay		100	Sept–Oct	Kremer (1994)
Chesapeake Bay	76	60	Aug–Sept	Purcell *et al.* (2001)
Long Island estuaries	159	142	Sept	McNamara *et al.* (2010)
Black Sea	304	184	Aug–Sept	Vinogradov *et al.* (1989) Shiganova *et al.* (2001)
Limfjorden	867	312	Aug–Sept	Riisgård *et al.* (2007) Riisgård *et al.* (2012)
Kiel Bight	505		Jun–Oct	Javidpour *et al.* (2009a) Javidpour *et al.* (2009b)
Wadden Sea	613	140	Jun–Nov	This study

Density and biomass remained relatively constant throughout the winter until March, when the density stayed constant but the bio-volume in terms of wet mass increased, suggesting a period of growth, but no spawning, as can also be seen in the length distribution.

The analysis of weekly averages of *M. leidyi* density in relation to abiotic factors reveals a significant relationship of ctenophore density with temperature. In the first half of the year ctenophore density increases exponentially with increasing temperature above ca. 13 °C, and levels off at temperatures > 17 °C. The second half of the year shows a different, more complicated pattern which is not possible to interpret using temperature alone. Here the temperature effect becomes negative two times. Probably other factors such as food availability (which was not measured) play a role here. A positive relationship between *M. leidyi* abundance and temperature is also observed in other areas, such as the Baltic Sea (Javidpour *et al.*, 2009b) and North American temperate waters (Kremer, 1994).

The presence of small ctenophores < 5 mm length was also significantly related to water temperature and was close to 100% probability at temperatures > 15 °C (Fig. 4.8). The first ctenophores < 5 mm were caught at a water temperature of 8 °C. This fits with the observation that in Narragansett Bay low egg production was observed at temperatures as low as 6 °C (Costello *et al.*, 2006).

In growth experiments, larvae of *M. leidyi* reached a length of 2 mm after 5–12 days (Sullivan and Gifford, 2007), which is roughly the minimum length at which they were caught with our sampling gear. As the eggs hatch between 20–24 h after spawning (Purcell *et al.*, 2001) and the first large batch of juveniles was observed in mid-May, spawning probably started 6–13 days earlier in the beginning of May. The mean water temperature in this period was 12.6 °C, which corresponds with observations on the start of spawning at ca. 12 °C in Chesapeake Bay (Purcell *et al.*, 2001). Egg production can resume within 2–4 days when favorable conditions occur (U. Båmstedt, unpublished data cited in Purcell *et al.*, 2001). The rapid increase in temperature in May preceding the first peak of *M. leidyi* in June might reflect such a period with favorable conditions. In both our models salinity was not a significant covariate. This might be because the minimum salinity observed (15) is still higher than that below which salinity becomes limiting to reproduction (Jaspers et al. 2011).

After each peak there was a rapid decrease in density, especially after the second one that only lasted one week. A possible cause might be predation. However, predators of *M. leidyi* larvae and juveniles such as *A. aurita*, *C. hysoscella* or *B. gracilis* were either absent or present in very low numbers. Intraspecific predation by adults of *M. leidyi* on juveniles has also been observed in high larval densities and at high water temperatures in the Baltic Sea (Javidpour *et al.*, 2009a,b) and this could be a factor contributing to the rapid decrease in density of larval and juvenile *M. leidyi*.

The highest mean density of *M. leidyi* recorded in this study was considerably higher than that observed in estuaries in its native range (Table 4.3), while the highest recorded mean bio-volume is almost equal or lower. The simultaneous occurrence of peaks in biomass with peaks in density in this study, is also observed in other studies in invaded areas in North-Western Europe, such as the

Limfjorden (Riisgård *et al.*, 2007) and Kiel Bight (Javidpour *et al.*, 2009a). At these peaks in biomass the population consists almost entirely of small (<20 mm length) ctenophores.

The large mesh size of 2 mm that was used for comparative reasons likely resulted in the loss of many of these small ctenophores. Thus our estimate of the density of *M. leidyi* during density peaks is likely even an underestimation of the actual density in the field.

The most recent studies on seasonal patterns of gelatinous zooplankton in the same area date back to the early 1980s. During that time *P. pileus* blooms occurred in spring with peak densities up to 17 ind m^{-3} (Van der Veer and Sadée, 1984). No such bloom of *P. pileus* was found in 2009, the highest mean density observed was only 3.5 ind m^{-3} in June. Scyphozoan density was so low that the filtering capacity of our sampling gear might be too low to sample them accurately, and a larger net such as an Isaacs-Kidd midwater trawl might be more suited to sample them. The small peak in *Beroe gracilis* density observed in 2009 occurred around the same time in the early 1980s, in early/mid June, after the first *P. pileus* bloom. *B. gracilis* is able to prey on especially small *M. leidyi* (Hosia *et al.*, 2011). Several times ingested *M. leidyi* juveniles inside *B. gracilis* were observed during this study (pers. obs.), but any quantitative estimation of the predation role of *B. gracilis* on *M. leidyi* is not possible at present.

In its native range, *M. leidyi* blooms are partially controlled by the ctenophore *Beroe ovata* Bruguiére, 1789, the scyphozoan Chrysaora quenquecirrha Desor and the butterfish Peprilus triacanthus Peck, 1804 (Purcell and Arai, 2001). These species do not occur in the Wadden Sea, and the local *Chrysaora-* (*C. hysoscella*) and *Cyanea* (*C. lamarckii* and *Cyanea capillata* L. 1758) species, of which the latter is a known predator of *M. leidyi* (Hosia and Titelman, 2011) were only present in very low numbers. At present the impact of *M. leidyi* on the ecosystem of the Wadden Sea is still unknown. Recent field and experimental work suggest that in the North Sea, the direct impact of *M. leidyi* as predator on fish eggs might be restricted and that the indirect effects via competition for food could be more important (Hamer *et al.*, 2011).

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Chapter 5

Changes in the gelatinous zooplankton community in the Dutch Wadden Sea following the invasion of the ctenophore *Mnemiopsis leidyi*

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Abstract

The ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 is a succesful, recent, macroplanktonic invader in European coastal waters, including the Dutch Wadden Sea. It occurs year-round in substantial numbers. The effect of *M. leidyi* on structure of the gelatinous zooplankton community in the Dutch Wadden Sea was studied by comparing data from before and after its introduction. Gelatinous zooplankton species composition in the western Wadden Sea was largely the same between 1980–1983 and 2009–2012. Only the hydromedusa *Eutonina indicans* (Romanes, 1876) was absent in recent years while *Cosmetira pilosella* Forbes, 1848 and *Margelopsis haeckelii* Hartlaub, 1897 were rare species observed only in 2009–2012. *M. leidyi* was present since 2006, with high densities every year in summer and autumn. Predation pressure by scyphomedusae, ctenophores, and hydromedusae on fish larvae and zooplankton in 1980–1983 was low because of low densities and a temporal mismatch between gelatinous zooplankton and their prey. Despite the decrease in abundance of scyphomedusae in recent decades, the introduction of *M. leidyi* and its large annual blooms have increased the overall importance of gelatinous zooplankton as predators in the western Wadden Sea, whereby *M. leidyi* is responsible for most of the predation pressure on mesozooplankton in the area. Rather than outcompeting local species, *M. leidyi* appeared to fill in a previously unoccupied niche in the pelagic western Dutch Wadden Sea in late summer and autumn.

Introduction

The invasion of non-native organisms can have major effects on local communities and ecosystems (Elton, 1958; Williamson, 1996). The most frequent vectors of marine invasions are shipping and aquaculture (Molnar *et al.*, 2008). Many organisms that get transported to other areas do not survive the journey, or perish because environmental conditions in the new environment are outside of their tolerance range. Of the ones that do survive, few establish themselves and even less become truly invasive (Williamson, 1996). Often, however, there is a period in which an introduced organism is present in low abundance. During this "lag" period, many introduced species go undetected. These lags can be caused by slow initial population growth, unsuitable environmental conditions, or a fitness deficit where the organism has to adapt to the new environment (Crooks *et al.*, 2001). There are many different hypotheses as to why and how an introduced species becomes invasive (Ricciardi *et al.*, 2013); including: propagule pressure; the number of individuals introduced; release from competition, predation, or parasitism; changes in environmental conditions; or the existence of an "empty" niche in the introduction area.

Assessing the effects of an invasive species requires detailed knowledge of the state of the ecosystem before the invasion (Blossey, 1999). For example, detailed knowledge on the ecosystem structure and function of lakes in the United States prior to invasion by the zebra mussel *Dreissenia polymorpha* (Pallas, 1771) allowed a detailed assessment of changes in food web organisation and ecosystem activity (Miehls *et al.*, 2009a,b).

In the Wadden Sea, an important fuelling station for migrating birds and an important nursery area for various fish species (Wolff, 1983), several successful invasions of bivalve species have been observed, e.g., the sand gaper *Mya arenaria* Linnaeus, 1758, Pacific oyster *Crassostrea gigas* (Thunberg, 1793) and razor clam *Ensis directus* (Conrad, 1843) (Beukema and Dekker, 1995; Wolff *et al.*, 2005; Troost, 2010; Dekker and Beukema, 2012). In 2006, the occurrence of an invasive ctenophore, *Mnemiopsis leidyi* A. Agassiz, 1865 was confirmed in Dutch coastal waters including the Wadden Sea (Faasse and Bayha, 2006; Tulp, 2006). *M. leidyi* is one of the recent successful invasive species in northern European waters. It has been reported from Sweden (Hansson and Kiørboe, 2006), Germany (Javidpour *et al.*, 2006; Boersma *et al.*, 2007), Denmark (Tendal *et al.*, 2007), the Baltic Sea (Javidpour *et al.*, 2006), Poland (Janas and Zgrundo, 2007) Norway (Oliveira, 2007). *M. leidyi* is an opportunistic planktonic predator, feeding on a wide range of different zooplankton prey such as copepods and their nauplii, bivalve veligers, barnacle nauplii, fish larvae and eggs (Cowan and Houde, 1992; Purcell *et al.*, 1994; Javidpour *et al.*, 2009b; Granhag *et al.*, 2011); therefore, competition with other gelatinous zooplankton species that have similar diets is possible.

For the western Wadden Sea data on the gelatinous zooplankton species composition and seasonal patterns prior to the *Mnemiopsis leidyi* invasion are available. Substantial quantitative published and unpublished baseline information exists from several macroplankton surveys in the 1980s (Van der Veer and Sadée, 1984; Van der Veer, 1985; Daan, 1986; Kuipers *et al.*, 1990). During that pe-

riod, a variety of hydromedusae and scyphomedusae species were present and the most abundant gelatinous zooplankton species was the ctenophore *Pleurobrachia pileus* (O. F. Müller, 1776), predated upon by *Beroe gracilis* Künne, 1939 (Van der Veer and Sadée, 1984). At present, *Mnemiopsis leidyi* has established itself as a macroplanktonic invader in the Dutch Wadden Sea, with a year-round occurrence in substantial numbers (Van Walraven *et al.*, 2013). In the period 2009–2012 macroplankton samples were taken using similar methods in the same area as during the 1980–1983 studies.

The goal of this paper was to investigate the potential impact of the invasion by *Mnemiopsis leidyi* on the gelatinous zooplankton community in the Dutch Wadden Sea by comparison of the patterns in the 1980–1983 period with those in recent years (2009–2013). The specific goals were to evaluate:

1. whether the introduction of *M. leidyi* was followed by a shift in species composition, seasonal pattern of occurrence, and abundance of the gelatinous zooplankton community;
2. whether the introduction of *M. leidyi* increased competition for food and, if so, during which periods of the year; and
3. the current and possible future ecological impact of *M. leidyi* in the Wadden Sea system.

Material and methods

Sampling

Surveys were conducted in the western Dutch Wadden Sea (Fig. 5.1) during 1980–1983 and 2009–2012 . All samples were collected in tidal gullies ranging from 5–15 m in depth at current velocities >20 cm s^{-1} from an anchored ship or from a jetty. For 1982 and 1983 complete data sets are available and the data from these years were used as a baseline against which to compare the 2009–2012 species composition, seasonal patterns, and abundance of gelatinous zooplankton. Water temperature and salinity during the two sampling periods were obtained from a nearby station from which surface water temperature and salinity were measured daily (Van Aken, 2008a,b). In 2009–2012, water temperature and salinity were also measured at the surface once during each zooplankton net-haul with a hand-held conductivity meter (WTW cond 330i, WTW, Welheim, Germany, accuracy ± 0.1 unit).

Sampling in the 1980s took place weekly at one or more of several stations in the Marsdiep tidal basin from February to December in 1982 and from April to December in 1983 (Figure 1). Oblique hauls were made with plankton nets made of polyamide plankton gauze (2-mm mesh size) with an opening of 0.7 m^2, a length of 5 m, a porosity of 0.59, and a total surface area of 12 m^2 (definitions according to Smith *et al.*, 1968). A flow meter mounted in the opening of the net was used to estimate the volume of water filtered. Samples collected in the 1980s were preserved in a 4% formaldehyde solution and photographed submerged

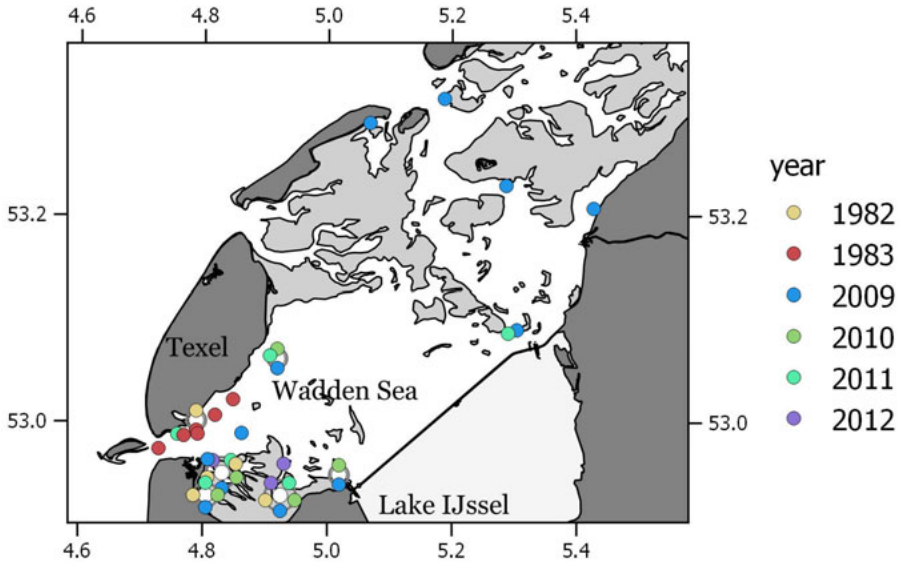

Figure 5.1: Stations in the western Wadden Sea sampled in this study, together with the years for which the station was sampled. Dark grey represents land, light gray intertidal area, white is subtidal.

in seawater in a flat basin with a black background. Organisms were identified, counted, and the lengths of a subsample of ca. 100 organisms were measured from a projection of the slide photographs using a ruler. More details on sampling methodology can be found in Van der Veer and Sadée (1984) (1982 data) and Kuipers *et al.* (1990) (1983 data). Raw data on ctenophores, scypho¬medusae, and hydromedusae from this period were derived from Van der Veer and Sadée (1984); Kuipers *et al.* (1990); and unpublished data (H. van der Veer and R. Daan). For 1982, colour-slide images of the original samples were available and used to confirm the species identifications for that period.

Recent sampling was done from January–December in 2009, during the second half of the year in 2010, from April–August in 2011, and from April–October in 2012 (Figure 1). Sampling frequency varied from weekly to monthly depending on availability of the research vessels in the sampling area as most of the sampling was carried out when the vessels were at station waiting for non-related research to be carried out on the tidal flats. After collection, the cod end of the net was emptied in a bucket and the contents were either identified to species level, counted, and individual sizes measured directly on board using a submerged 1 mm sieve directly after collection, when high numbers of juvenile ctenophores occurred, fixed on board using the trichloroacetic acid (TCA) method (Adams *et al.*, 1976), applied for fixation of quantitative samples as described in Van Walraven *et al.* (2013). Sampling protocol and methods corresponded with those during the period 1982–1983, except for 2012. In 2012, the gear was changed from a 2-mm mesh size to a 1-m diameter net with 1 mm mesh size, a circular opening of 0.78 m^2,

and a length of 5 m. Water flow through the net was estimated using a flow meter mounted in the net opening.

For each haul, all individuals were identified to species level based on Greve (1975) for ctenophores, Holst (2012a) for scyphomedusae and on Russell (1953), Cornelius (1995b,a) and Schuchert (2012) for hydromedusae. For each species numbers per sample were converted into densities (individuals m^{-3}). For each date, the mean (± SE) number per haul (as ind. m^{-3}) was then calculated. Sizes were measured to the nearest mm on a measuring board with 1 mm scale except for large scyphozoa which were measured to the nearest cm and juvenile ctenophores, which were measured to the nearest mm submerged in a petri dish using a binocular microscope and ocular micrometer.

Predation estimates

Potential for competition for food with other gelatinous zooplankton by *Mnemiopsis leidyi* was studied by estimating and comparing the species-specific prey consumption rates in a manner similar to that used in Limfjorden, Denmark (Riisgård *et al.*, 2012). For each of the four most abundant species in the period 2009–2012, the daily clearance rate for an average sized individual F_{ind} was estimated using predator size–clearance rate relationships for copepods and/or copepodites obtained from several published sources (Table 5.1). This was done for all years. If needed, animal biovolume was estimated from measured lengths using allometric relationships from other studies (Table 5.2).

Population clearance rates F_{pop} of the four most abundant species were calculated by multiplying individual clearance rate for an average-sized individual F_{ind} with the population density (D, ind m^{-3}). Assuming that population clearance rates F_{pop} are independent of temperature and prey density and that the water is well mixed, the time it takes for a population of a predator species to reduce the concentration of a prey species by half ($t_{\frac{1}{2}}$) was estimated following Riisgård *et al.* (2012) as follows:

$$t_{\frac{1}{2}} = \frac{ln2}{F_{pop}} \tag{5.1}$$

If the half-life time was longer than three weeks, it was considered negligible as half-life time then exceeds the average generation time for copepods (Riisgård *et al.*, 2012). Data analyses and modelling were perfomed using R (R Core Team, 2014). Figures were made using the ggplot2 package (Wickham, 2009).

Table 5.1: Size–clearance rate relationships used in clearance rate estimation F_{ind} = clearance rate per individual (l^{-1} ind d^{-1}), V = live volume in ml, D = diameter in mm, L = polar length in mm, H= bell height in mm.

nr	Species	Relationship	Reference
1	*Pleurobrachia pileus*	$F_{ind} = 0.2L^{1.9}$	Møller *et al.* (2010)
2	*Mnemiopsis leidyi*	$F_{ind} = 2.64V$	Riisgård *et al.* (2012)
3	*Aurelia aurita*	$F_{ind} = 0.0073D^{2.1}$	Møller and Riisgård (2007)
4	*Sarsia tubulosa*	$F_{ind} = e^{2.75ln(H)-0.95} * 24/1000$	Hansson and Kiørboe (2006)

Table 5.2: Biometric conversions for gelatinous zooplankton used in this study. V = live volume in ml, D = diameter in mm, OA = Oral−Aboral length in mm, TL = total length in mm, WW = wet weight in g, DW = dry weight in mg.

Species	Conversion	Reference
Pleurobrachia pileus	$TL = 1.25D$	Van der Veer and Sadée (1984)
Pleurobrachia pileus	$TL_{fresh} = TL_{form.fixed}/0.80$	Van der Veer and Sadée (1984)
Pleurobrachia pileus	$TL_{fresh} = TL_{TCAfixed}/0.81$	Van Walraven *et al.* (2013)
Mnemiopsis leidyi	$OA_{fresh} = OA_{TCAfixed}/0.81$	Van Walraven *et al.* (2013)
Mnemiopsis leidyi	$V_{fresh} = 0.0226OA_{fresh}^{1.72}$	Riisgård *et al.* (2007)

Results

Environmental conditions

The average water temperature values were remarkably consistent between years. Mean weekly water temperature typically showed a minimum in January– February and a maximum in July–August (Fig. 5.2). The lowest single observed winter water-temperature was −1.5 °C in January 1982 but winter water temperatures usually exceeded 2 °C. The highest single observation of summer water-temperature was 22.0 °C in July 1983 but the average seldom exceeded 19 °C.

Salinity was quite variable between months and between years, most weekly averages ranged between 24 and 31. The lowest values (<23) were observed in January–February 1982 with the next lowest values (<24) being observed during May to July 1983.

Species composition

Seventeen species of gelatinous zooplankton were caught: 4 ctenophores, 5 scyphomedusae, and 8 hydromedusae (Table 5.3). Three species of hydromedusae were only observed on one or two days in some of the years during the 2009−2012 period: *Aequorea vitrina* Gosse, 1853 was caught every year in very low numbers with a maximum of 3 individuals per year. The earliest observation in the year was on April 8, 2010 and the last on August 14, 2012. Fourteen individuals of *Cosmetira pilosella* Forbes, 1848 were caught on August 11, 2011 only. A single specimen of *Margelopsis haeckelii* Hartlaub, 1897 was collected on June 21, 2011.

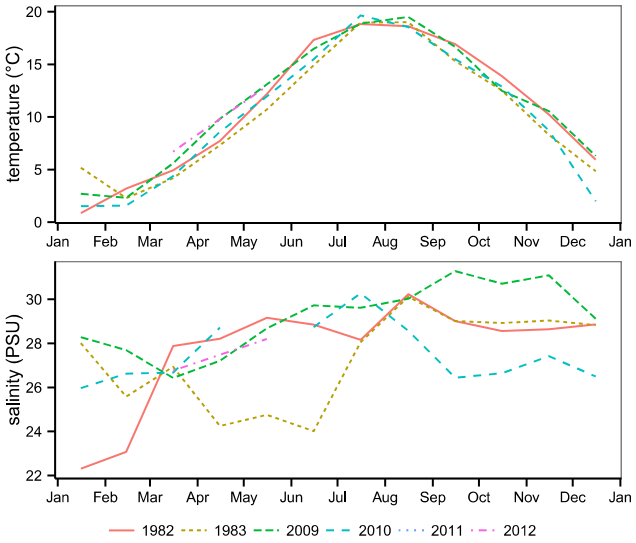

Figure 5.2: Mean monthly surface seawater temperature (°C, top) and salinity (PSU, bottom) at the NIOZ jetty in the western Wadden Sea for the studied years.

The Hydromedusae *Clytia hemisphaerica* (Linnaeus, 1767) and *Eucheilota maculata* Hartlaub, 1894 were present, often in high numbers, throughout the sampled period. These species could not be distinguished from each other on the scanned slide photographs from 1981–1982 and were grouped together under "Leptothecata". The black spots on the outer wall of the stomach of *E. maculata* (Cornelius, 1995b) were visible in most, but not all TCA-fixed samples (Adams *et al.*, 1976). If absent, the presence and number of marginal cirri at each tentacle bulb was used as a distinguishing feature. The thecate hydroid *Eutonina indicans* could be distinguished by its elongated gastric peduncle, also in fixed samples.

Abundant athecate hydroids on the 1982 slide photographs that were previously identified as *Bougainvillia ramosa* (Van Beneden, 1844), now accepted as *Bougainvillia muscus* (Allman, 1863) were assigned to *Nemopsis bachei* L. Agassiz, 1849 for this study based on the presence of ribbon-like folds that extended along the radial canals more than two-thirds towards the bell margin (Schuchert, 2012). In the fixed samples these structures were sometimes missing, in this case the species was identified by checking for the presence of the two small capitate tentacles at the tentacle bulbs (Schuchert, 2012). No individuals of *B. mucus* were detected in any of our samples.

Five species of scyphomedusae were found: *Aurelia aurita* (Linnaeus, 1758), *Chrysaora hysoscella* (Linnaeus, 1767), *Cyanea capillata* (Linnaeus, 1758), *Cyanea lamarckii* Péron and Lesueur, 1810 and *Rhizostoma octopus* (Linnaeus, 1758). All species were observed in all years in both periods except for *C. capillata* which was only found in 2009–2012.

With respect to the ctenophores, *Mnemiopsis leidyi* was found only in 2009–2012. Of the other species, the cydippid ctenophore *Pleurobrachia pileus* was present in all years as was its predator, the beroid *Beroe gracilis*. *Beroe cucumis* Fabricius, 1780 was identified from the samples in 2011. Because small (<20 mm) individuals of the two *Beroe* species were very difficult to distinguish especially from the fixed samples and photographs, their abundances are combined as *Beroe* spp.

Table 5.3: Overview of gelatinous zooplankton species caught in the surveys, per year. *other years uncertain

Species		Years present					
Hydromedusae		1982	1983	2009	2010	2011	2012
Aequorea vitrina	Gosse, 1853	x	x	x	x	x	x
Clytia hemisphaerica	Linnaeus, 1767	x	x			x	x
Eucheilota maculata	Hartlaub, 1894	x	x	x	x	x	x
Cosmetira pilosella	Forbes, 1848						
Eutonina indicans	Romanes, 1876	x	x				
Margelopsis haeckelii	Hartlaub, 1897					x	
Nemopsis bachei	L. Agassiz, 1849	x	x	x	x	x	x
Sarsia tubulosa	M. Sars, 1835	x	x	x	x	x	x
Scyphomedusae							
Aurelia aurita	Linnaeus, 1758	x	x	x	x	x	x
Chrysaora hysoscella	Linnaeus, 1767	x	x	x	x	x	x
Cyanea capillata	Linnaeus, 1758			x	x	x	
Cyanea lamarckii	Péron & Lesueur, 1810	x	x	x	x	x	x
Rhizostoma octopus	Linnaeus, 1758	x	x	x	x	x	x
Ctenophores							
Beroe cucumis	Fabricius, 1780					x*	
Beroe gracilis	Künne, 1939	x	x	x	x	x	x
Mnemiopsis leidyi	A. Agassiz, 1865			x	x	x	x
Pleurobrachia pileus	O. F. Müller, 1776	x	x	x	x	x	x

Most hydromedusae species were caught at sizes >1 mm (Table 5.4). The five most abundant species, *Eutonina indicans*, *Sarsia tubulosa* (M. Sars, 1835), *Nemopsis bachei*, *Clytia hemisphaerica* and *Eucheilota maculata* (the last two combined here as Leptothecata) showed distinct seasonal patterns (Fig. 5.3). *S. tubulosa* was the first species appearing, generally in April with an earliest observation on March 24 in 2011; it reached its peak abundance in May and was never caught after June. *E. indicans* showed a similar seasonal pattern as *S. tubulosa* but was only observed in the 1980s. The ocombined leptothecates group appeared in June and peaked generally in mid-summer. They remained present until the end of the year. *N. bachei* was also a summer species; in most years its peak abundance was reached in July. In 2009 and 2011 *N. bachei* was already observed at the end of April. Two other species (*Cosmetira pilosella* and *Margelopsis haeckelii*) were only observed during a single month (August and June 2011, respectively).

There were strong seasonal patterns in abundance of scyphomedusae (Fig. 5.4). *Cyanea capillata* was not observed in the 1980s, and infrequent observeed in April/May only in 2009–2011. *Aurelia aurita* and *Cyanea lamarckii* appeared at about the same time around March with the abundances of both species peaking

in late April/early May. *Chrysaora hysoscella* and *Rhizostoma octopus* appeared next, peaking in August/September. Peak abundances of *A. aurita* were an order of magnitude lower in 2009–2012 than in 1982 and 1983. When species appeared in the catches, they had a minimum size of 4 or 5 mm (Table 5.4).

Ctenophores

The ctenophore *Pleurobrachia pileus* was always present very early in the year, often present at the start of the sampling period (Fig. 5.5). In 1982 and 1983 there were two peaks in abundance: a spring peak in May and a second autumn peak with an order of magnitude lower densities in October/November. In 1982 and 1983 *P. pileus* was the most abundant gelatinous zooplankton species. The timing and magnitude of the spring bloom remained relatively constant throughout the sampled period. In 2009–2012 the timing of the spring bloom was the same as in the 1980s, but the peak densities were an order of magnitude lower. In recent years, the autumn bloom was much less pronounced and often absent. In most years the predatory *Beroe* species peaked approximately one month after the *P. pileus* peak, in June. In 2011 *Beroe* reached its highest densities in autumn. *Beroe cucumis* was only observed in 2011 when large beroid ctenophores were collected which had the branching of the gastrovascular system characteristic of *Beroe cucumis* (Greve, 1975). In 2009 and 2012 *Beroe* larger than 30 mm (the published maximal length for *B. gracilis*, Greve (1975) were also observed but the branches where not visible. *Mnemiopsis leidyi* was absent in the 1980s but present in all recent years. In most years, it was present in low densities throughout the year, and bloomed in August–October at densities an order of magnitude higher than those of *P. pileus*. The year 2009 was an exception with the *M. leidyi* bloom already underway in June.

Prey consumption

Potential food consumption was estimated for the four most abundant species: the hydromedusae *Sarsia tubulosa*, the scyphomedusae *Aurelia aurita*, and the ctenophores *Pleurobrachia pileus* and *Mnemiopsis leidyi*.

During 1981–1983, *Pleurobrachia pileus* had the highest water-column clearance rates. In line with the trends in abundance, clearance rates showed a peak in spring (Fig. 5.6). Year-to-year differences in clearance rate were large. Maximum rates in spring varied between 0.2 and 0.8 m^3 per m^3 per day. Clearance rates of *A. aurita* were an order of magnitude lower than those of *P. pileus*, with highest average rates during summer of 0.003 ± 0.004 m^3 per m^3 per day and clearance rates in *S. tubulosa* were even lower than those of *A. aurita*, with highest average rates in spring of 0.001 ± 0.002 m^3 per m^3 per day. Any impact of gelatinous zooplankton on the rest of the plankton community was restricted to the first half of the year, after July the combined clearance rate of the three species became very low.

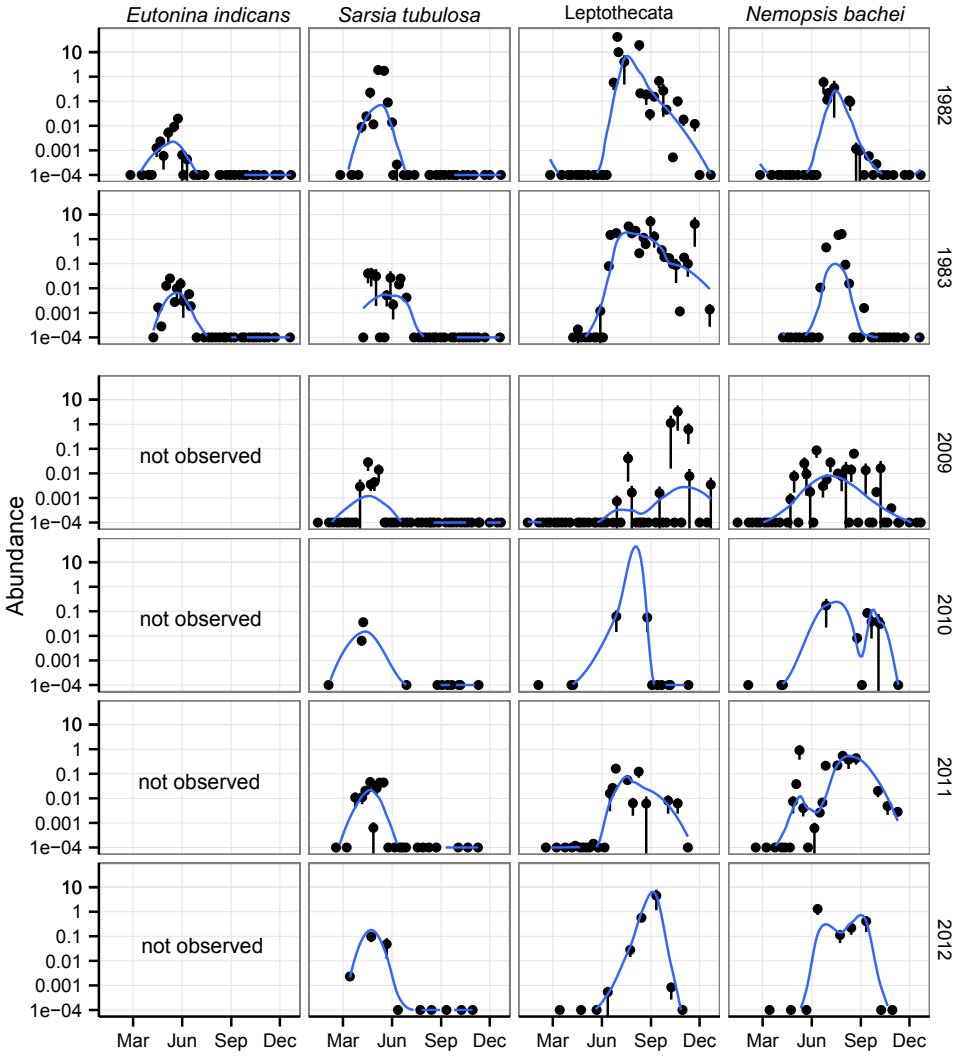

Figure 5.3: Mean weekly densities (n m^{-3} + $1e^{-4}$) with standard errors for *Sarsia tubulosa*, *Eutonina indicans*, *Leptothecata* and *Nemopsis bachei* averaged over all stations. A LOESS smoother (span=0.6) is added for interpretation.

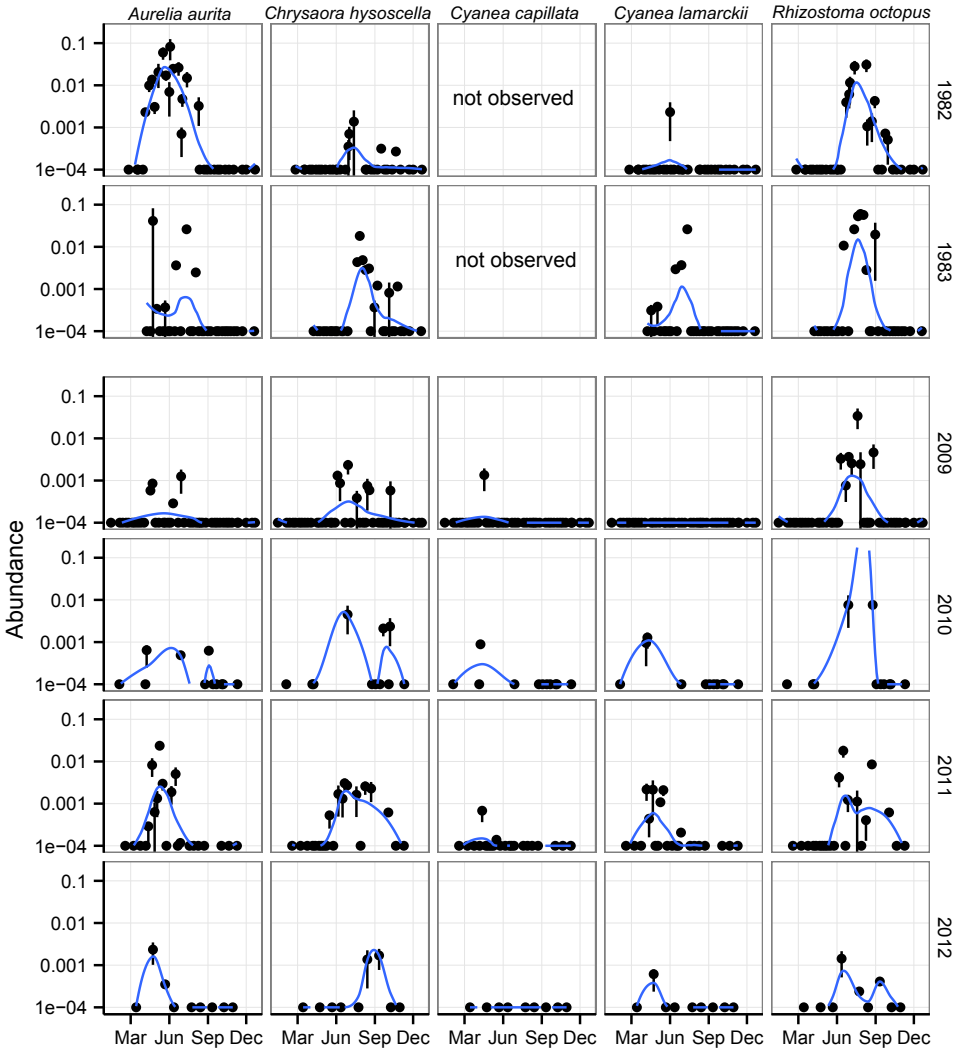

Figure 5.4: Mean weekly densities (n m^{-3} + $1e^{-4}$) with standard errors for scyphomedusae averaged over all stations. A LOESS smoother (span=0.6) is added for interpretation.

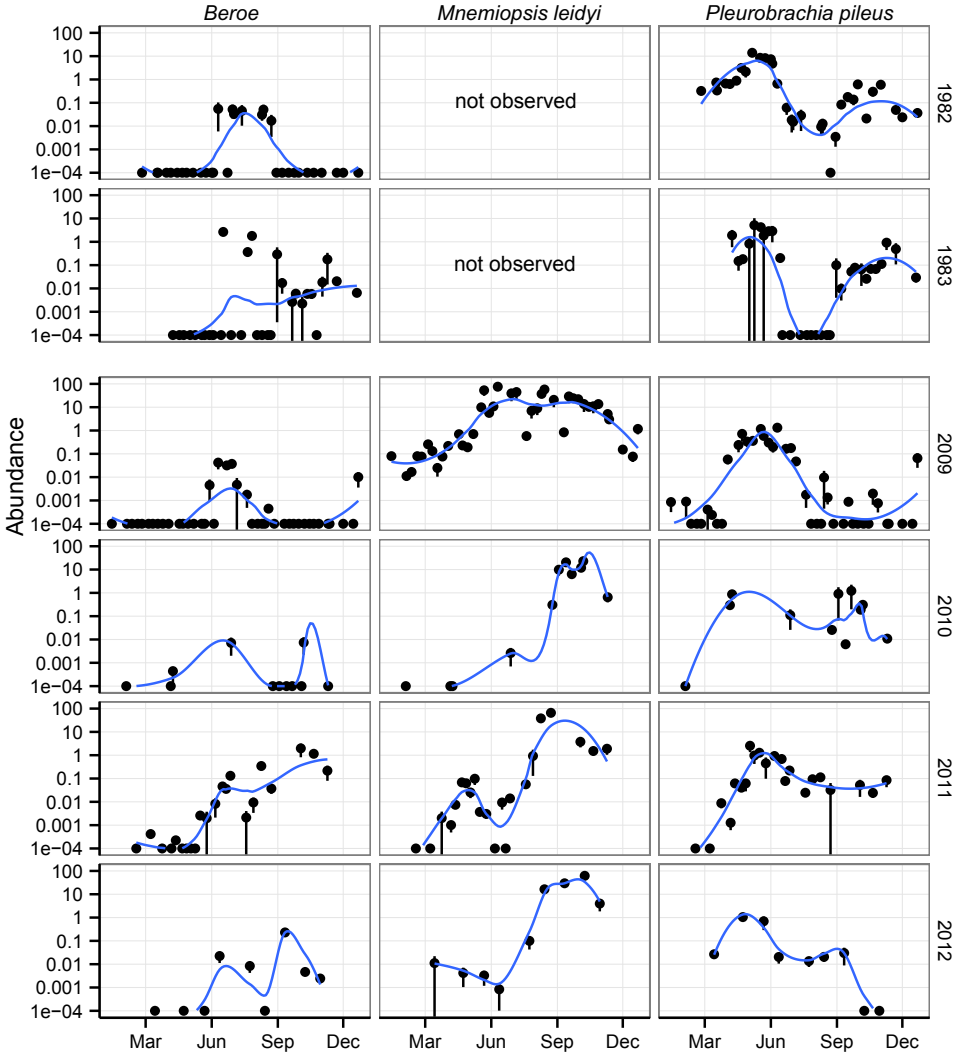

Figure 5.5: Mean weekly densities (n m^{-3} + $1e^{-4}$) with standard errors for *Pleurobrachia pileus*, *Mnemiopsis leidyi* and *Beroe spp.* averaged over all stations. A LOESS smoother (span=0.6) is added for interpretation.

Table 5.4: Mean, minimum and maximum sizes (mm) of gelatinous zooplankton species in 2009–2012. Sizes are bell diameters for thecate hydromedusae and scyphomedusae, bell height for athecate hydromedusae, polar length for *Beroe* spp. and *Pleurobrachia pileus* and oral–aboral length for *Mnemiopsis leidyi*.

Species	min	mean	max
Aequorea vitrina	19	35	55
Aurelia aurita	4	104	305
Beroe spp.	1	9	70
Chrysaora hysoscella	4	45	150
Clytia	2	7	14
Cosmetira pilosella	7	12	16
Cyanea capillata	5	22	108
Cyanea lamarckii	5	52	350
Eucheilota maculata	1	5	21
Mnemiopsis leidyi	1	6	92
Nemopsis bachei	1	6	16
Pleurobrachia pileus	1	5	49
Rhizostoma octopus	4	76	340
Sarsia tubulosa	1	9	23

Period 2009–2012

For *Sarsia tubulosa*, *Aurelia aurita* and *Pleurobrachia pileus* clearance rates during 2009–2012 were lower than those during 1981–1983 (Fig.5.6). Highest clearance rates of *Pleurobrachia pileus* occurred in spring with average rates of 0.013 ±0.042 m^3 per m^3 per day. Clearance rates of *A. aurita* were highest in summer with average rates of 0.0001 ± 0.0005 m^3 per m^3 per day and clearance rates for *S. tubulosa* were highest in summer with average rates of 0.0001 ± 0.0002 m^3 per m^3 per day.The main difference between both periods was the additional high clearance rate by *Mnemiopsis leidyi*. *M. leidyi* was the most important gelatinous zooplankton species in terms of predation pressure on the zooplankton. *M. leidyi* population clearance rates were highest in late summer–autumn and in 2009 also in spring. The periods of high daily clearance rates of *M. leidyi* were often of a short duration of one or a few days.

The highest maximum clearance rate F_{pop} of *M. leidyi* was slightly higher than 1 m^3 per m^3 per day. Estimated zooplankton half-life times showed the same trend.

Using the clearance rate estimates, zooplankton half-life time was longer than three weeks for all species studied except for *Rhizostoma octopus*, *Pleurobrachia pileus*, and *Mnemiopsis leidyi* with that of *R. octopus* being lower than three weeks only on a single day; hence, it was not shown (Fig. 5.7). In spring 2011 and 2012, *P. pileus* was still the most important zooplankton predator, but the introduction of *M. leidyi* has caused there to be even higher total predation rates in summer and autumn. For most of the period investigated, the *M. leidyi* population had the highest predation rate of any species studied. Contrary to the 1981–1983 period,

in 2009–2012 the combined impact of gelatinous zooplankton was not restricted to the first half of the year but was highest in the second half of the year, mainly in late summer to early autumn.

Species	max F_{pop}	date
Aurelia aurita	9.4	2011-05-09
Chrysaora hysoscella	0.8	2009-07-20
Cyanea capillata	0.7	2010-04-08
Cyanea lamarckii	0.6	2011-05-17
Mnemiopsis leidyi	1047.5	2009-08-19
Nemopsis bachei	6.7	2012-06-13
Pleurobrachia pileus	320.6	2012-05-23
Rhizostoma octopus	37.2	2009-07-21
Sarsia tubulosa	1.2	2012-04-24
Leptothecata	2.8	2009-10-27

Table 5.5: Maximum values of daily average clearance rate (F_{pop}, L per m^3 per day) for each species in 2009–2012.

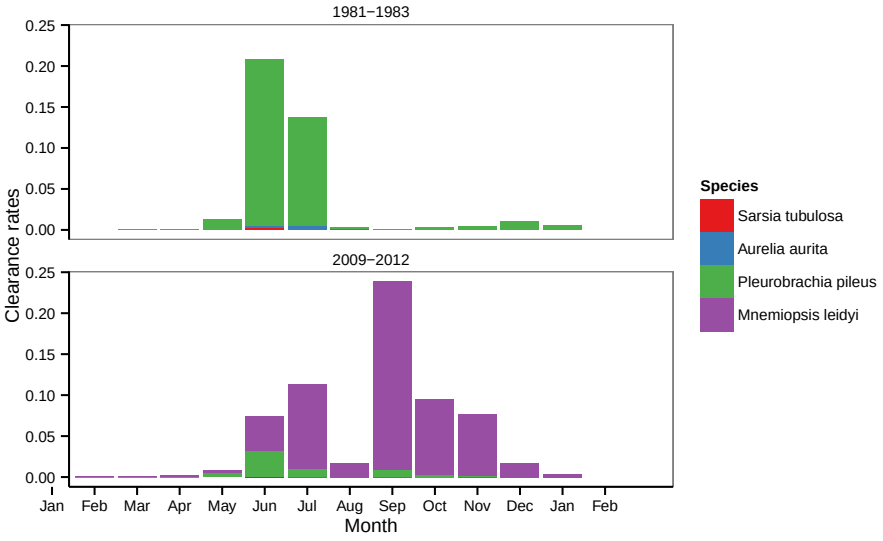

Figure 5.6: Mean monthly population clearance rates F_{pop} in m^3 per m^3 per day by *Sarsia tubulosa*, *Aurelia aurita*, *Pleurobrachia pileus* and *Mnemiopsis leidyi* in the western Wadden Sea as estimated using the size–clearance rate relationship found in Table 5.1. For 1981 only data on *Aurelia aurita* and *Pleurobrachia pileus* were available.

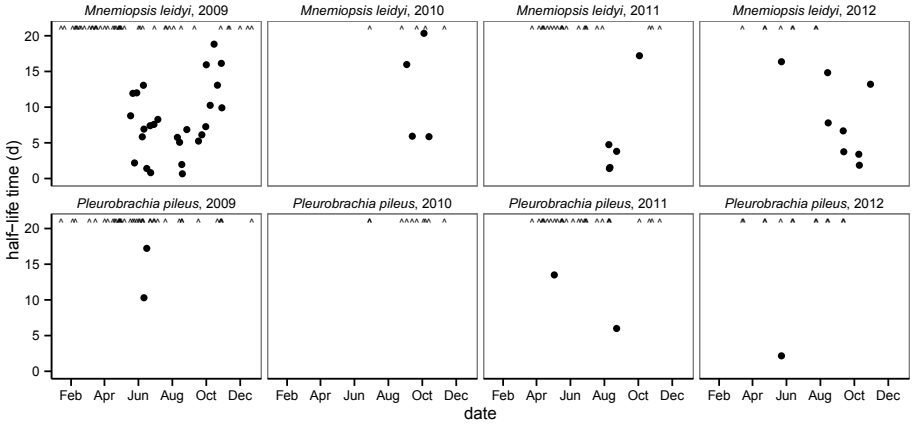

Figure 5.7: Mean daily zooplankton half-life times of *Mnemiopsis leidyi* and and *Pleurobrachia pileus* in 2009–2012 estimated from the daily average F_{pop} for the species. Values higher than 21 days are indicated by '^'.

Discussion

A comparison between the 1980s and the present situation is complicated by the fact that after the 1980s, several biotic and abiotic parameters of the western Wadden Sea have changed: a reduction in riverine N and P inputs (van Raaphorst and de Jonge, 2004) was followed by a decrease in primary production (Cadée and Cadée-Coenen, 2002) and the average annual seawater temperatures have risen by 1.5 °C, an increase which can be found in all seasons (Van Aken, 2008b). These and other factors have lead to changes in the timing and magnitude of phytoplankton blooms (Philippart *et al.*, 2010), changes in phenology of various organisms (Philippart *et al.*, 2003; Dekker and Beukema, 2014; Van Walraven *et al.*, 2015) and a reduction of heterogeneity, trophic structure and nursery function (Eriksson *et al.*, 2010; Van der Veer *et al.*, 2015). A large number of invasive species have been found since the 1980s as well (Wolff *et al.*, 2005). All of these changes are known to influence gelatinous zooplankton abundance, seasonal pattern, and species composition (reviewed in Purcell, 2012; Lucas and Dawson, 2014).

An analysis of 50 years of Scyphozoa catches in a kom-fyke, a passive fishing gear, in the same area revealed that catches decreased following the decrease in primary production in the 1980s but these variables could not be linked to each other as abundances were highly variable (Van Walraven *et al.*, 2015). In the same study, phenology of Scyphozoa changed; *Aurelia aurita* appeared earlier in the year in response to increased winter seawater temperatures and *Chrysaora hysoscella* was present for a longer period in autumn following warmer summers. Similar changes in phenology in response to temperature changes may also have occurred in other gelatinous zooplankton taxa.

Species composition

A comparison of the species composition over time requires observations obtained and identified in a similar way, preferably by standardised protocols. Sampling methods in this study were almost the same for both periods and species identification was done following the same procedure.

Several difficulties were encountered, however, during taxonomical identification of the samples. For example, Van der Baan (1980a) had problems with high abundances of the thecate hydroid *Clytia hemisphaerica* dominating the catches and making it impossible to identify the small thecate hydroids to species level in the formaldehyde-fixed samples. In this study the method used for analysing the 1982 catches (identifying, counting and measuring from slide photographs) could not be used to identify most of the thecate hydromedusae to species level. For that, analysis of fresh or preserved samples using a dissection microscope is necessary.

Nemopsis bachei was originally found in The Netherlands only in the former Zuiderzee, before it was closed off and turned into a freshwater lake in 1932, with a latest sighting in 1928 (Wagenaar Hummelinck, 1954). In 1993, numerous medusae were caught in the Eastern Scheldt estuary and were first identified as *Bougainvillia* sp., but later concluded to be *Nemopsis bachei* based on the presence of the two small capitate tentacles on each tentacle bulb (Faasse and Ates, 1998). In 2002 the

species was also found in the Dutch Wadden Sea (Tulp, 2002). The authors of the two papers argue that the species was likely present after 1932, but misidentified as *Bougainvillia* species. The findings in this study confirm that *N. bachei* was at least present in 1982 and 1983 in the western Wadden Sea.

Systematics within the genus *Beroe* are confusing (Bayha *et al.*, 2004) and in need of revision. In 2011 large individuals of *Beroe cucumis* were observed for the first time in the surveys. *B. cucumis* has been observed before in the Wadden Sea (Holsteijn, 2002). Another species of *Beroe*, *Beroe ovata* sensu Mayer 1912, was observed in Danish waters in 2014 Shiganova *et al.* (2014). The main difference between *B. cucumis* and *B. ovata* seems to be the lack of anostomosing diverticulae in the first species (see Appendix B). Anostomosing diverticulae were never observed in *Beroe* from the western Wadden Sea by the authors of this study. Eastern Atlantic *Beroe cucumis* is likely a different species from that occurring in the Mediterranean which has also been named *Beroe ovata* sensu Chun (Mills *et al.*, 1996).

Although species composition differed between years and between the two periods due to presence/absence of rare species, the overall species composition was rather similar with the same species being abundant. The most significant difference is the presence of high densities of *Mnemiopsis leidyi* in recent years. The first confirmed sightings in the western Wadden Sea are from 2006 (Faasse and Bayha, 2006). It might have been present earlier, misidentified as *Bolinopsis infundibulum* (O.F. Müller, 1776) in 2002 (Holsteijn, 2002) or even as early as 1992 (Faasse and Ligthart, 2007). Although large catches of ctenophores were observed in summer in the fish catches of the NIOZ kom-fyke fish trap long-term monitoring programme in some years before 2006 (H. Witte, NIOZ Royal Netherlands Institute for Sea Research, Texel, pers. comm.) the identity of these ctenophores is unknown.

Seasonal abundance

A comparison of seasonal occurrences of hydromedusae and scyphomedusae with those described by Russell (1953, 1970) and Van der Baan (1980b,a) is presented in Table 5.6.

Hydromedusa

Russell (1953) mentions that *Eucheilota maculata* usually occurs in late summer and autumn. Its hydroid is found in Dutch coastal waters (Cornelius, 1995a; Vervoort and Faasse, 2009). *Clytia hemisphaerica* medusae were released in spring and summer off Plymouth according to Russell (1953), and Van der Baan (1980a) mention that the species was abundant near the lightvessel "Texel" from June to January, with peak catches in autumn. In this study peak catches of small Leptothecata were in summer, with the highest densities found in 1982. *Eutonina indicans* was not observed in the recent years. It is found in the Wadden Sea and other Dutch coastal waters infrequently (Vervoort and Faasse, 2009; Tulp, 2001). Ates (2003) gives an overview of sightings of *E. indicans* in The Netherlands and

also notes the sporadic occurrence of the species; often with no records for several decades.

Polyps of *Sarsia tubulosa* are known to occur in Dutch coastal waters, including the western Wadden Sea (Vervoort and Faasse, 2009). The seasonal pattern of *S. tubulosa* in the western Wadden Sea was the same as described by Cornelius (1995a) in British waters.

This study confirmed that *N. bachei* was at least present in 1982–1983. Thiel (1967) assumed that *N. bachei* arrived in Europe in the mid 19th century from the Atlantic coast of North America attached to ship hulls, but the scarcity of sightings makes it impossible to confirm this. The solitary polyps of *N. bachei* are very inconspicuous and have not been found in the field. However, since small medusae of 1 mm length are also found in the Wadden Sea, the polyp is likely to be found in or near the area. According to Schuchert (2012) the reproductive season of the species is summer–autumn, but in this study it was already observed in April in 2009 and 2011 in relatively high spring temperatures.

Two species of hydromedusae were only observed in 2011. *Margelopsis haeckelii* is known from the Wadden Sea already, the medusae as well as the pelagic polyp stage (Vervoort and Faasse, 2009). *Cosmetira pilosella* has not yet been recorded in the plankton of Dutch coastal waters, but several individuals were caught in 1961-1966 from the lightvessel "Texel" located approximately 20 nm offshore Texel island in the North Sea by Van der Baan (1980a).

Scyphomedusae

Van der Baan (1980a) studied the seasonal patterns of ephyrae and medusae of scyphomedusae extensively based on plankton samples taken in 1961–1966 from the lightvessel "Texel". She found post-ephyrae (2–10 mm diameter) of *Cyanea lamarckii* from November–June in high densities, post-ephyrae of *Aurelia aurita* in some years in January but most years after March. Small post-ephyrae of *Chrysaora hysoscella* were much less numerous and post-ephyrae of *Rhizostoma octopus* were never observed, although Van der Baan (1980b) mentions that they are often observed in the Wadden Sea.

The minimum size of all five scyphomedusae species found in this study (Table 5.4) is at or close to the size of newly released ephyrae found by Holst (2012b). This means that medusae are likely to be produced in or close to the western Wadden Sea and not only transported there by advection as in the central Baltic Sea (Barz *et al.*, 2006). Experimental work on polyps of the species observed here shows that they can survive in the environmental conditions encountered in the western Wadden Sea (Holst and Jarms, 2010; Holst *et al.*, 2007). Only for *Aurelia aurita* have the polyps been found in Dutch coastal waters (Lindeyer and Gittenberger, 2011).

Ctenophores

For ctenophores, information on seasonal occurrence is present for the German Bight (Greve and Reiners, 1988). The timing of the spring bloom of *Pleurobrachia*

pileus in this study was similar to that in the adjacent German Bight. In most years there was a clear succession visible with *Beroe* peaking one month after the *Pleurobrachia pileus* bloom, as is also observed in the North Sea (Greve and Reiners, 1988; Greve *et al.*, 2004). Hosia *et al.* (2011) showed that *B. gracilis* can prey on invasive *M. leidyi.* If *B. gracilis* would be able to successfully prey- and reproduce on a diet of *M. leidyi* densities of the predator should be much higher in late summer– autumn, when high densities of *M. leidyi* occur. Fig. 5.5 however does not suggest that *B. gracilis* has increased in the recent years. Only in 2011 there was a clear increase in *Beroe* density in autumn following the *M. leidyi* bloom. In that period *Beroe* densities were an order of magnitude higher than those of its prey *P. pileus*.

The seasonal pattern of *M. leidyi* in the western Wadden Sea normally includes blooms in summer and autumn, comparable to the seasonal pattern in Narragansett Bay and Long Island estuaries in the native range (Kremer, 1994; McNamara *et al.*, 2010). In several studies *M. leidyi* reproduction was shown to be related to temperature (Costello *et al.*, 2006; Robinson and Graham, 2014) and increased winter survival of *M. leidyi* combined with early warming of the water in spring could lead to earlier blooms of *M. leidyi*, as has been observed in the species' native range as well (Costello *et al.*, 2006; McNamara *et al.*, 2010; Robinson and Graham, 2014). This consequently means increased overlap with the annual zooplankton spring bloom, as well as spawning periods of bivalves (Philippart *et al.*, 2014) and fish (Van der Veer, 1985). The seasonal pattern of *M. leidyi* in 2009 shows that earlier blooms have happened at least once already in the western Wadden Sea. As shown in Van Walraven *et al.* (2013), salinity is unlikely to influence the seasonal pattern or abundance of *M. leidyi* within the range of salinity (22–32) observed in the Wadden Sea. In contrast, salinity can be much lower in Scandinavian waters and limits the establishment of *M. leidyi* there (Haraldsson *et al.*, 2012).

The introduction of *Mnemiopsis leidyi* does not seem to have affected species composition or seasonal occurrence of most other gelatinous zooplankton species; however, some impact on the abundance of the other ctenophores (especially *P. pileus*) cannot be excluded. During the 2009–2012 period, the size of the peak abundance of *P. pileus* in spring seemed to be much smaller than in the period 1982–1983, suggesting some form of species interaction. In Kertinge Nor, Denmark, high abundances of *Aurelia aurita* appear to limit the establishment of *M. leidyi* in a shallow cove (Riisgård *et al.*, 2010). Densities of *A. aurita* in the Wadden Sea were much lower than in Kertinge Nor, so it is unlikely that *A. aurita* is limiting *M. leidyi* here.

Impact on the zooplankton

Information on the standing stock of zooplankton in the western Wadden Sea was not available for 1982 and 2009–2012, therefore it was not possible to compare changes in grazing pressure with changes in zooplankton concentrations. The available data on gelatinous zooplankton abundance and density in the western Wadden Sea in the 1980s was used in several studies which investigated grazing pressure on zooplankton by the most abundant species of ctenophores, scyphome-

Table 5.6: Occurrences of hydromedusae and scyphomedusae in this study compared with published studies from Dutch waters (Van der Baan, 1980a,b) and around the United Kingdom (Russell, 1953). s=scarce (not every year), c=common (every year)

		Month											
Species	Study	j	f	m	a	m	j	j	a	s	o	n	d
Sarsia tubulosa	Russell (1953)				c	c	c	s	s	s	s		
	Van der Baan (1980a)	s	c	c	c	c	s						
	this study			c	c	c	c						
Clytia sp.	Russell (1953)	c	c	c		c	c	c	c	c	c	c	c
	Van der Baan (1980a)						c	c	c	c	c	c	
	this study								c	c	c	c	
Eucheilota maculata	Russell (1953)							c		c	c	c	
	Van der Baan (1980a)						c	c	c	c	c	c	
	this study				s	s			s				
Aequorea vitrina	Russell (1953)	s			s	s	s		s	s		s	s
	Van der Baan (1980a)	s	s			s	s	s	s	s	s	s	s
	this study				s	s			s				
Nemopsis bachei	this study				c	c	c	c	c	c	c	s	s
Cosmetira pilosella	Russell (1953)			s	c	c	c	c	c	s	s		
	Van der Baan (1980a)			s	s	s					s		
	this study				s								
Aurelia aurita	Russell (1970)	s	s	s	c	c	c	c	c	c	s		
	Van der Baan (1980b)	s		s	c	c	c	c	s				
	this study				c	c	c			s			
Chrysaora hysoscella	Russell (1970)					c	c	c	c	c	s		
	Van der Baan (1980b)					c	c	c	c	c	s	s	
	this study						c	c	c	c	c		
Cyanea lamarckii	Russell (1970)	s			s	s	s	s					
	Van der Baan (1980b)	c	c	c	c	c	c					c	c
	this study				c	c	s						
Cyanea capillata	Russell (1970)	s	s	s	c	c	c	c	c	c	s		
	Van der Baan (1980b)												
	this study				s	s							
Rhizostoma octopus	Russell (1970)	s	s	s	s	s	s	s	c	c	c		
	Van der Baan (1980b)									s	s	s	
	this study						c	c	c	c	c		

dusa and hydromedusa respectively, all of which found that the impact of this predation on zooplankton stocks was low to moderate. Densities of most of these species were similar or even lower in 2009–2012 suggesting that they are even less important as predators now as they were in the 1980s. Daan (1986) determined ingestion rates and growth rates of *Sarsia tubulosa* in experiments and used these to investigate growth and grazing pressure of *S. tubulosa* in situ. He found that growth appeared to be optimal and predation impact on the zooplankton stocks was negligible. Hansson and Kiørboe (2006) estimated size-based clearance rates of *S. tubulosa* feeding on different prey types in the lab. They used the data on *S. tubulosa* size and density from (Daan, 1986) to estimate prey mortality rates in the field and reached similar conclusions. When the method of (Hansson and Kiørboe, 2006) is used to estimate clearance rates for *S. tubulosa* in 1982 and 2009-2012 a similar low impact on zooplankton stocks is found.

The predation pressure by *Aurelia aurita* on zooplankton in the western Wadden Sea was found to be low to moderate in 1981 and 1982 (Van der Veer, 1985; Van der Veer and Oorthuysen, 1985). As densities of *A. aurita* were lower in recent years, the clearance rates of the *A. aurita* population were also lower, as expected.

Predation on zooplankton by *Pleurobrachia pileus* was investigated using different methods in the 1980s. Specifically, Van der Veer and Sadée (1984) estimated the food demand of *P. pileus* in the western Wadden Sea based on ctenophore density and observed growth (here an increase in mean diameter) in the field. They suggest that *P. pileus* is important as a predator in May, as in this month the *P. pileus* population would need to consume half of the zooplankton standing stock daily in order to sustain the observed growth rate. Following up, Kuipers *et al.* (1990) supplemented field sampling with digestion time experiments and stomach content analysis of caught animals, which were used to estimate prey consumption rate. Based on this they estimate a daily mortality rate of less than 0.01 for copepods and copepodites by *P. pileus* predation. This is more than an order of magnitude lower than the estimates of population clearance rate using the same data and the length–clearance rate relationship of (Møller *et al.*, 2010). A reason for this may be that Kuipers *et al.* (1990) based their consumption rates on stomach content analysis of field-caught ctenophores. Stomach contents were very low in the study of Kuipers et al., with the majority of stomachs being empty. This suggests that regurgitation may have occurred during fixation (Van der Veer, 1985), capture or handling (Larson, 1987; Chandy and Greene, 1995).

In the 1980s, the seasonal period of highest abundance of the most common species *A. aurita* and *P. pileus* showed little overlap with that of several potential prey species. The immigration of flatfish larvae was earlier (Van der Veer, 1985) and the start of the mesozooplankton bloom occurred when gelatinous predator densities were already decreasing again (Kuipers *et al.*, 1990).

With *Mnemiopsis leidyi* present the seasonal pattern in zooplankton predation rates was different. While the seasonal pattern of *P. pileus* abundance in 2009–2012 was similar to that observed in the 1980s, the average abundance and thus clearance rate were much lower, making the species less important a predator in the recent period. Densities of other native zooplankton species were similar or even lower in 2009–2012 than in the 1980s, suggesting that they as well

are less important as predators now as they were in the 1980s. Here it is shown that *M. leidyi* is now the most important zooplanktivorous predator in the area. Furthermore, the period of highest clearance rates and thus predation rates on zooplankton, has shifted from late spring to late summer and autumn (Figure 5.6). A recent study has shown that in spring as well as autumn, the Marsdiep area of the western Wadden Sea is an important habitat for zooplanktivorous fish species, including commercial species such as sprat *Sprattus sprattus* (Linnaeus, 1758), herring *Clupea harengus* Linnaeus, 1758, pilchard *Sardina pilchardus* (Walbaum, 1792), and anchovy *Engraulis encrasicolus* (Linnaeus, 1758), which are in turn an important food item for birds, fish, and mammals in the area (Couperus *et al.*, 2016). High predation rates by *M. leidyi* in the autumn period might cause decreased food availability for zooplanktivorous fish and then have cascading effects through the food web.

In a nearby area invaded by *Mnemiopsis leidyi*, Limfjorden, mesozooplankton was virtually absent at the highest combined clearance rates of *Aurelia aurita* and *M. leidyi* (Riisgård *et al.*, 2012). In that area mesozooplankton stocks did not show the annual peaks in abundance that were present in previous years before the introduction of *M. leidyi*. Clearance rates of *M. leidyi* in the western Wadden Sea were comparable to those found in Limfjorden and on several days even higher, suggesting a comparable grazing pressure of the invasive ctenophores on mesozooplankton in this area. This is also reflected in the zooplankton half-life times estimated from the population clearance rates (Fig. 5.7) which were often less than three weeks, which is an average generation time for copepods (Gillooly, 2000). On the days with the shortest half-life times it is likely that *M. leidyi* was controlling the zooplankton stocks on several occasions.

As Purcell (2009) and Riisgård *et al.* (2012) note, their clearance rate estimates are conservative. The relationship between clearance rate and total length of Colin *et al.* (2010) leads to slightly lower estimated clearance rates for a given length when oral-aboral length is assumed to be 60% of total length (L. van Walraven, unpublished data). When using the relationship between oral-aboral length and clearance rate of Granhag *et al.* (2011), clearance rates are up to 7 times higher, depending on ctenophore size (Figure 5.8). Actual predation pressure of *M. leidyi* on zooplankton in the area might be even higher than estimated here.

Larval and juvenile *M. leidyi* feed mainly on microzooplankton (Sullivan and Gifford, 2004), but growth rates of microzooplankton-fed *M. leidyi* decrease when the larvae reach a length of 4–5 mm (Sullivan and Gifford, 2007). By predating on microzooplankton-feeding mesozooplankton, adult *M. leidyi* can decrease competition for their juveniles as well (McNamara *et al.*, 2013b).

The introduction of *M. leidyi* has lead to an increase in predation pressure of gelatinous zooplankton compared to the 1980s, especially in late summer and autumn, a period when in the 1980s grazing pressure by gelatinous zooplankton was very low. This means that, rather than out-competing local species, it appears that *M. leidyi* has found an empty or under-utilised niche in the Wadden Sea pelagic ecosystem. The concept of invasive species occupying a previously-vacant niche is well known in invasion ecology (Wilson and Turelli, 1986; Hierro *et al.*, 2005) under different names (Catford *et al.*, 2009).

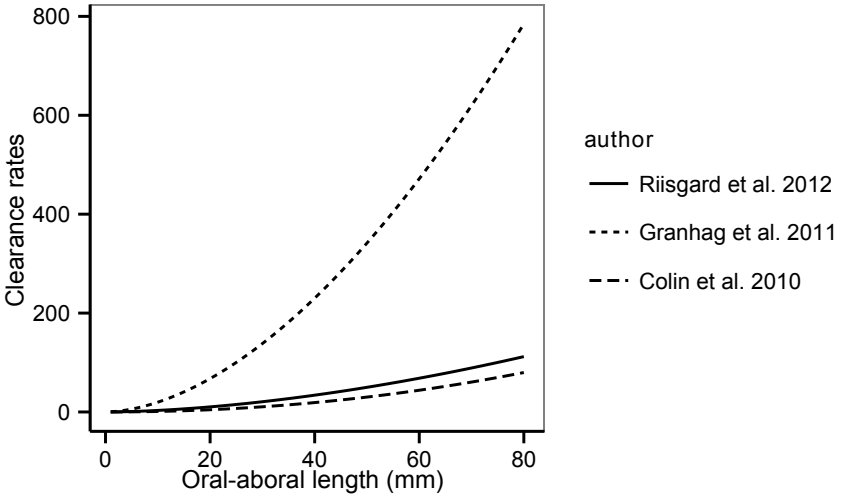

Figure 5.8: Different relationships between *Mnemiopsis leidyi* oral-aboral length and individual clearance rates in liters per individual per day from other studies. Riisgård *et al.* (2012) is used in this study. For Colin *et al.* (2010) who used total lengths instead of oral-aboral length, oral length was assumed to be 60 % of total length.

In the Wadden Sea, one of the main examples of an invasive species occupying an empty niche is the introduced razor clam *Ensis directus*. This species occupies exposed parts of the intertidal zone that emerge infrequently. This habitat used to have a low density and biomass of macrozoobenthos, but could be used by *E. directus* which is well adapted to frequent digging and moving in sand (Beukema and Dekker, 1995; Dekker and Beukema, 2012). Something similar might be happening with *M. leidyi*; an under-used niche in the area as a zooplanktivore in summer and autumn.

Acknowledgements

The authors would like to thank the crew of the research vessels 'Stern' and 'Navicula' for their assistance during sampling, Ewout Adriaans, Bram Fey, Hein de Vries, Tony van der Vis and Wim Jan Boon and all the students that assisted over the years. Henk Hobbelink digitised the slide photographs. L. van Walraven was supported by the GELMESOZOOPLANKTON grant from Stichting Deltares Foundation. The 2012 survey was funded by the Netherlands Organization for Scientific Research (NWO) via Project 839.08.241 of the National programme Sea and Coastal Research (ZKO).

Box: Stomach content analysis of *Mnemiopsis leidyi*

MSc project Michiel van Harten

Methods

Stomach contents of field-caught *Mnemiopsis leidyi* were extracted by injecting 10 ml of sterile filtered seawater into the stomach cavity to flush out stomach contents. Ctenophores were collected in the western Wadden Sea in June–August 2011. Stomach contents were analysed visually using a binocular microscope (n = 45), but for part of the samples the filtrate was collected and vacuum filtered over a 20 µm nylon filter (n = 16). Filters were stored at -80 °C for molecular analysis of bivalve species in the stomach contents. To investigate presence of bivalve larvae in the plankton 19 samples were collected by filtering 2 L of surface seawater through a 50 µm filter and collecting the residue. Primers for six bivalve species *(Crassostrea gigas*, *Mytilus edulis*, *Cerastoderma edule*, *Mya arenaria*, *Macoma balthica* and *Ensis directus*) were used in the PCRs for species identification (see Philippart *et al.*, 2014, for methodology).

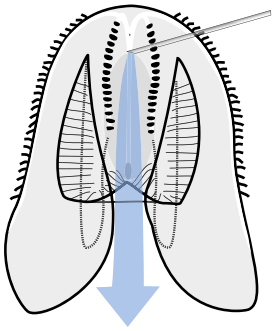

Figure 5.9: Injection of syringe needle to flush out stomach contents with 10 ml of filtered seawater.

Results and discussion

Average density of bivalve larvae in the plankton was 2.46 ind L^{-1}. Five bivalve species were identified from the water samples; *(C. gigas*, *M. edulis*, *C. edule*, *M. arenaria* and *E. directus*. 56 % of visually analysed and 80 % of molecular analysed stomach contents contained bivalve larvae. DNA of the same species found in the water samples was found in the stomach content, except for *C. edule*.
This shows that it is possible to identify bivalve larvae to species level in *M. leidyi* stomach contents and that the species does indeed feed on bivalve larvae in the western Wadden Sea as well.

Part II

Invasion success of *Mnemiopsis leidyi* investigated

Chapter 6

Mechanisms behind the metabolic flexibility of an invasive comb jelly

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Abstract

Mnemiopsis leidyi is an invasive comb jelly which has successfully established itself in European seas. The species is known to produce spectacular blooms yet it is holoplanktonic and not much is known about its population dynamics in between. One way to gain insight on how *M. leidyi* might survive between blooms and how it can bloom so fast is to study how the metabolism of this species actually responds to environmental changes in food and temperature over its different life-stages. To this end we combined modelling and data analysis to study the energy budget of *M. leidyi* over its full life-cycle using Dynamic Energy Budget (DEB) theory and literature data.

An analysis of data obtained at temperatures ranging from 8 to 30 °C suggests that the optimum thermal tolerance range of *M. leidyi* is higher than 12 °C Furthermore *M. leidyi* seems to undergo a so-called metabolic acceleration after hatching. Intriguingly, the onset of the acceleration appears to be delayed and the data do not yet exist which allows determining what actually triggers it. It is hypothesised that this delay confers a lot of metabolic flexibility by controlling generation time.

We compared the DEB model parameters for this species with those of another holoplanktonic gelatinous zooplankton species (*Pelagia noctiluca*). After accounting for differences in water content, the comparison shows just how fundamentally different the two energy allocation strategies are. *P. noctiluca* has an extremely high reserve capacity, low turnover times of reserve compounds and high resistance to shrinking. *M. leidyi* adopts the opposite strategy: it has a low reserve capacity, high turnover rates of reserve compounds and fast shrinking.

Introduction

The comb jelly *Mnemiopsis leidyi* (A. Agassiz 1865) is native to the Atlantic coast of the United States of America (USA) and invasive in Europe (Costello *et al.*, 2012). It was first accidentally introduced to the Black Sea in the 1980s from the Gulf of Mexico population. Then it was accidentally introduced to the North Sea from the north-east coast of the USA (Reusch *et al.*, 2010).

At present, this species seems to be well established in European waters and its potential predatory effects on fish recruits and competition with zooplanktivorous fish species are of major concern. Thus there is a continued interest to elucidate its impact on local community structure and functioning. A rather essential problem in this context is to understand and predict size and maturity structure of populations throughout the season because this relates to recruitment dynamics, timing of blooms and strategies to wait out bleak periods.

The first step is to understand individual energy budgets which can later be used for extrapolations to the population level. (Reeve *et al.*, 1989) proposed a first carbon budget for this species, but many new datasets have since been acquired. And importantly, more advanced methods exist now to perform mass and energy balancing on different types of field and laboratory data, namely the Dynamic Energy Budget (DEB) theory. In this study we combine modelling and data analysis to study in depth the energy budget of *M. leidyi* over its full life-cycle using the DEB theory and literature data.

The DEB theory is a theoretical framework which specifies metabolic organisation of all living organisms from uni-cellular bacteria to whales. The theory offers a precise formulation of which metabolic processes need to be represented in order to fully quantify the energy/mass budget of an animal (Kooijman, 2001; Sousa *et al.*, 2008). The formulation of these processes and how they interact make up the standard DEB model which applies to animals (Kooijman, 2010, 2012). Parameters for this model have been estimated for many different animal species (see e.g. Kooijman and Lika, 2014, for a recent overview). A number of those species seem to accelerate their metabolism after birth (Kooijman, 2014), i.e. they seem to increase metabolic rates in connection with their development. This metabolic, or type $\mathcal{M}$, acceleration is newly introduced to the eco-physiological literature but might well concern a large number of animal species which warrants us mentioning it here.

In this study we estimate a set of DEB model parameters on the basis of available literature data. The data all originate from different geographical locations (US and Europe) and years (1976–2014), were obtained at different temperatures (8–31 °C) and were measured in different ways (different types of weight or length measurements). By comparing the different data to a same reference (DEB model predictions) we provide an overview of this species' energy budget. We further obtain a set of DEB model parameter values based on a sub-set of the data. Last, the new findings are discussed relative to our current understanding of the biology and ecology of *M. leidyi* and compared with that of another gelatinous holoplanktonic species, the purple mauve stinger *Pelagia noctiluca* (Forskål, 1775).

Setup of the DEB model

We herein briefly outline the setup of the standard DEB model extended to deal with the type $\mathcal{M}$ metabolic acceleration introduced in the precedent section. The way metabolism is conceptualized according to this model is presented in Fig. 6.1A. Arrows represent energy fluxes expressed in J d^{-1}. The energy fluxes are functions of the DEB model parameters which are listed in Table 6.1. The equations are listed in Section A.1, Online Appendix. Boxes represent variables: energy in food E_X (J), energy in faeces E_P (J), energy in reserve E (J), structural volume V (cm^{-3}), cumulated energy invested in maturation E_H (J) and in reproduction E_R (J). The state variables of the individual are E, V, E_H and E_R. The total mass of the individual is the sum of the mass of reserve and of structure respectively. We assume that energy invested into reproduction is immediately released into the environment in the form of gametes and so neglect its potential contribution to the total mass. Furthermore maturity itself has no mass or energy: the investment dissipates into the environment.

The model specifies energy allocation to all of the processes over the full life-cycle as shown in Fig. 6.1 B where we illustrate how important metabolic events are situated along a maturity gradient (thick black line). Maturity is quantified as the cumulated amount of energy in joules invested into the process of maturation. Age zero is defined as the start of development (conception) and, by definition, E_H = 0 J. Energy investment into maturation encompasses any expenses linked to tissue differentiation, i.e. re-organisation of body structure from tentaculate to lobate form. This is different from growth which can be conceptualized as synthesis of more of the same.

Energy invested into growth is fixed into the biomass of the organism (with some overheads), but energy invested in maturation is oxidized as metabolic work making it more difficult to quantify in practice. Nonetheless it can be quantified and it can even represent a substantial part of the energy budget (Augustine *et al.*, 2011; Mueller *et al.*, 2012).

Birth occurs at maturity level $E_H = E_H^b$ and is defined as the moment external assimilation is initiated. This probably coincides with hatching. Puberty, is defined as the onset of adult egg production and occurs by definition at maturity level $E_H = E_H^p$. At puberty the individual stops allocating to maturation ($dE_H/dt = 0$) and starts allocating towards reproduction. This metabolic switch in energy allocation is represented by the grey circles in Fig. 6.1. The state variable ER (thick red line, Fig. 6.1B) represents cumulated energy invested into reproduction. The model specifies energy allocation to metabolism over the full life-cycle of the organism because we include a maternal effect rule where the reserve density ([E] = E/V) of the neonate at birth is taken equal to that of the mother at spawning (Kooijman, 2009). Maturity levels E_H^b and E_H^p are model parameters.

As mentioned in the introduction, a number of animal species were found to accelerate their metabolism right after birth. We include the possibility for type $\mathcal{M}$ metabolic acceleration to occur between the maturation window $E_H^s \leq E_H \leq E_H^j$, where E_H^s and E_H^j (J) are the maturity levels where acceleration begins and ends respectively. They are also both model parameters. During metabolic acceleration

both the energy conductance ($\dot{\nu}$ in cm d^{-1}) and the maximum surface-area specific assimilation rate ($\{\dot{p}_{Am}\}$ in J cm $^{-2}$d^{-1}) must be multiplied by a shape correction function (Eq. A.1.1, Section A.1, Online Appendix). We refer the reader to Kooijman et al. (2011) and Kooijman (2014) for more details concerning the shape correction function.

Maturity level E_H^j can be constructed as a metabolic metamorphosis after which $\dot{\nu}$ and $\{\dot{p}_{Am}\}$ take on permanent values. The ratio of pre- and post-metamorphic values is given by the acceleration factor $s_{\mathcal{M}} = L_j/L_s$ where L_s and L_j represent structural lengths at the start of the metabolic acceleration and at metamorphosis respectively.

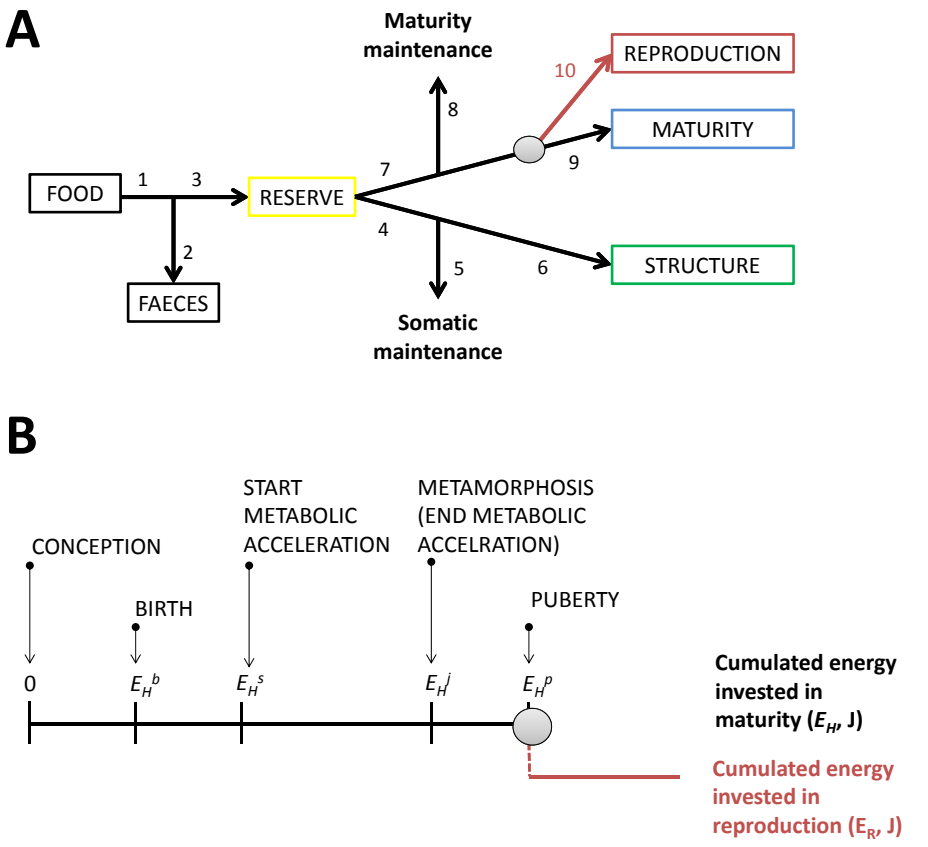

Figure 6.1: Full life-cycle DEB model of the invasive comb jelly, *Mnemiopsis leidyi*. (A) DEB model scheme. Boxes: variables; arrows: energy fluxes J d^{-1}. The numbers correspond to equations given in Subsection A.1, Online Appendix. The state variables are food (E_X, J), faeces (E_P, J), structure (V, cm^3), reserve (E, J), maturity (quantified by the cumulated energy invested in maturation, E_H, J), and investment into reproduction (quantified as cumulated energy invested into reproduction E_R , J). Maturity and somatic maintenance costs are energy sinks. Grey circle: metabolic switch between the processes of maturation and of reproduction. (B) Definition of the different life stages in the DEB model. Between conception ($E_H = 0$) and birth ($E_H = E_H^b$) there is no external assimilation. Assimilation is switched on after birth. After birth at maturity level $E_H = E_H^s$ the organism starts metabolic acceleration which ends at metamorphosis ($E_H = E_H^j$). After puberty ($E_H = E_H^p$), i.e. the lobate adult stage, allocation towards maturation stops and allocation towards reproduction starts.

Data, parameter estimation and modelling choices

We use the co-variation method of parameter estimation as defined in Lika *et al.* (2011) where data are divided into two categories: zero- and uni-variate. The former consist of single values which have no dependant variable (such as age at birth). The latter consist of sets of dependant and independent variables (such as length as function of time or weight as function of length).

In order to estimate parameters we collected literature data on the growth, reproduction, development, dioxygen consumption, and ammonia and carbon dioxide excretion. All of the data which we used are collected in Section A.2, Online Appendix.

DEB theory assumes that parameters are specific to individuals although values might be similar between members of a same population or a same species. The data that exist come from a variety of different research institutions, were obtained at different years and were performed on very different populations. Thus differences between methods and ambient salinity in combination with genetic differences between populations would most likely make it impossible to fit everything with a same parameter set.

We deemed that the most rigorous first approach was to avoid making an a priori choice about which data to include or exclude from parameter estimation so at first we attempted to simultaneously fit the model to all of the different datasets that we compiled (ca. 64 datasets, see Online Appendix A.2). Our objective was to obtain DEB model parameters by fitting the DEB model to the maximum number of different types of datasets with the minimum number of parameters (Occam's razor) in order to calculate the dynamic energy budget of *M. leidyi* over it's whole life-cycle in as parameter sparse and general way as possible.

Since we aimed for generality, certain species-specific characteristics were not included into the model such as the fact that ctenophores are simultaneous hermaphrodites (but see Harbison and Miller, 1986, for exceptions) and sometimes start reproducing right after birth (Hirota, 1972; Jaspers, 2012; Jaspers *et al.*, 2013; Martindale, 1987). We simply assumed reproduction starts at puberty and that energy invested into reproduction is converted to eggs with some overheads k_R. We further neglected any costs associated with the male function, but this will be addressed in more depth in the discussion.

All of the important modelling assumptions which were made when fitting the model to the data are listed in the following subsections. The consequences of these choices with respect to our results are discussed in the next section Results and discussion.

Feeding and condition

The scaled functional response f is used as a quantifier for food level. It takes value 0 for no assimilation to 1 for maximum assimilation for a given size. Some of the data were obtained from individuals sampled in the field. In that case individuals probably experienced a variety of food levels. Other data were obtained under

laboratory conditions and then the authors usually aimed for ad libitum feeding conditions.

To avoid increasing the number of parameters which need to be estimated, and because we did not have more detailed information on the nutritional status of the individuals, we assumed that f = 1 for all of the datasets. We further assumed that the organisms were always in equilibrium with their environment meaning that the reserve density is constant: d[E]/dt = 0.

The scaled reserve density $e = [E]/[Em]$ is a proxy for the individual's nutritional status (or condition). $[E_m]$ = $\{\dot{p}_{Am}\}$ /$\dot{\nu}$ is the maximum reserve density. At equilibrium e = $f$which simplifies the equations substantially. Some of the studies also report clearance and ingestion rates. These types of measurements depend a lot on the type of food, the life stage and the experimental set-up and were not included in the current analysis. Nonetheless we still needed to specify some general digestion efficiency (κ_X) and faecation efficiency (κ_X^P) in order to compute contributions of assimilation overheads to total respiration and ammonium excretion rates in juveniles and adults. κ_X and κ_X^P are model parameters and were taken equal to 0.8 and 0.1 respectively after Lika *et al.* (2011).

The link between life-history and maturity levels

The reader is referred to Rapoza *et al.* (2005, Fig. 1) for an overview of the different life-stages: cydippid, transitional and lobate. Briefly *M. leidyi* hatches as a cydippid larvae with tentacles. During the transitional stage the tentacles regress and lobes begin to form. At the end of the transitional stage there are no more tentacles and the individual has fully formed lobes.

We need to link maturity levels to actual morphological developmental milestones if we want estimates for size and age at each milestone according to dynamic food and temperature (Augustine *et al.*, 2011). We took the maximum size at puberty reported in the literature: 30 mm total length from Reeve *et al.* (1989). However it is more complex to know at which developmental milestone metabolic metamorphosis occurs. The observed range of oral-aboral (OA) lengths at the end of the cydippid larval stage (transitional stage), is 2 mm (Jaspers et al., 2013) to 5 mm (Martindale, 1987). The age and OA length at end of the transitional stage (so when lobes are fully formed) is also food and temperature dependant: published values range from 6 mm (Sullivan and Gifford, 2004) to 10 mm (Rapoza *et al.*, 2005). During parameter estimation we tried to link metamorphosis either to the end of the cydippid larval stage or to the end of the transitional stage.

Lengths

Physical lengths, L_w (cm) are taken proportional to structural lengths L (cm), L = $V^{1/3}$: L_w = $L/\delta_{\mathcal{M}}$ where $L/\delta_{\mathcal{M}}$ is the shape coefficient which relates each type of length to the structural length. We included corrections for the different types of measurements. Three types of length measurements were reported in the data we analysed: OA, oral–statocyst (OS) and total lengths. Here, we do not distinguish between OA and OS. In reality, *M. leidyi* changes in shape during ontogeny in

addition to increasing its water content (Anninsky *et al.*, 2007; Rapoza *et al.*, 2005; Reeve *et al.*, 1989). One way to take changes in shape into account would have been to make $L/\delta_{\mathcal{M}}$ a function of maturity level (or structural length) such that it decreases or increases as the organism goes from one morphology to another. For simplicity we assume that $L/\delta_{\mathcal{M}}$ is constant for each dataset.

Hydration and weights

There are 8 ways which weight is quantified in the literature: (1) carbon mass, (2) nitrogen mass, (3) ash-free dry mass (AFDM), (4) organic matter content, (5) salt-free dry fraction, (6) dry mass (DM), (7) wet mass (WM) and (8) displacement volume. According to DEB theory, reserve and structure respect strong homoeostasis (in terms of elemental frequencies and chemical potential) even though both types of material comprise rich mixtures of monomers and polymers such water, ions, lipids, sugars and proteins (Kooijman, 2010). We modelled the water free fraction of organic compartments and assumed that this corresponded to measurements (3–5). We took the water-free C:H:O:N in both reserve and structure (as well as food and faeces) equal to 1:1.8:0.5:0.1 after Lika et al. (2011), Table 2. This gives molecular weights of water-free reserve and structure (w_E and w_V) equal to 23.2 g mol^{-1}. Chemical potentials allow converting energies to C-mol and we took μ_E = μ_V = 560 kJ mol^{-1} for reserve and structure respectively. We need to specify the chemical potential of food and faeces and took μX = 525 kJ mol^{-1} and μ_P = 480 kJ mol^{-1}, also after Lika *et al.* (2011).

Next we assume that the specific densities of hydrated reserve and structure are equal d_E^w = d_V^w = 1 g cm^{-3} and that both reserve and structure have the same level of hydration. Under the assumption that w_E = w_V and that both reserve and structure have the same amount of water, we can derive the specific density of the water free fraction of both compartments as d_E = d_V = δ_W d_V^w g cm^{-3} where δ_W is the observed AFDM/WM ratio. The literature reports different values for δ_W and in fact the level of hydration even changes during ontogeny. We assume that δ_W = 0.003 g g $^{-1}$ and convert dry weights to ash-free dry weights assuming that salt represents 91 % of the total dry mass after McNamara *et al.* (2013a). Last, we work with the very simple premise that δ_W is constant over ontogeny. This assumption can be relaxed by assuming that $d_E \neq d_V$ and/or $w_E \neq w_V$. Some of the DEB model parameters (found in Table 6.1) are sensitive to the hydration level. For instance, the parameter [EG] in J cm^{-3} quantifies the structure specific costs for growth and it is directly proportional to dV. In addition, it was recently shown that $\{\dot{p}_{Am}\}$, $[\dot{p}_M]$ (volume linked somatic maintenance in J d^{-1} cm^{-3}) as well as the maturity levels also seem to change by the same factor as d_V (Lika *et al.*, 2014b). But this coupling requires further study.

Dioxygen consumption and ammonia and carbon dioxide excretion

Dioxygen consumption as well as ammonia and carbon dioxide excretion follow from the mass balancing equations of the DEB model. Briefly, the predictions

are weighted sums of assimilation, dissipation and growth. The equations can be found in the appendixes of both Mueller *et al.* (2012) and Augustine *et al.* (2014a). We assumed that the contribution from assimilation was zero in all studies where organisms fasted before the measurements. The predictions assume that for each size class f = 1 so that mobilisation is not yet affected by the absence of food.

Reproduction rates

We assumed that low reproduction rates correspond to individuals of lesser condition after Kremer (1976a). In practice this meant fitting the model to the highest values of reproduction rates observed for each size on the assumption that food-deprived individuals of a given size will decrease (and perhaps cease) reproduction quite fast. The ambient salinity level was shown to impact reproduction rates (Jaspers *et al.*, 2011) and so we focus on the higher salinity ranges.

Temperature

We assume that all metabolic rates in a single individual are affected by temperature in the same way, so that a change in temperature amounts to a transformation of rates using the simple Arrhenius relationship (Kooijman, 2014): $\dot{k}(T) = \dot{k}(T_{ref})exp(\frac{T_A}{T_{ref}} - \frac{T_A}{T})$ where $\dot{k}$ is a rate and T, T_{ref} and T_A are the experimental, reference and Arrhenius temperatures (in Kelvin) respectively. The DEB model parameters with time in their dimension are standardized to T_{ref} = 293 K (20 °C) The Arrhenius relationship was used to correct all model predictions for rates and ages from T_{ref} to the experimental temperature. We assumed that T = 26 °C for data by Baker and Reeve (1974) based on the geographical location. However this was unfortunately not explicitly specified in the original manuscript.

Results and discussion

We found that differences in measurements between studies and populations were too great and it was not possible to fit all of the data listed in Online Appendix A, Section A.2 using a set of DEB models parameters in combination with the simplifying assumptions found in Sections 3.2–3.7.

We were able to obtain a satisfactory fit to a coherent subset of all of those data: namely its overall life history (see Table 6.2), observed weight against length relationships (Fig. 6.2A–C) and reproduction rates against size (Fig. 6.2D–F) as well as its growth (Fig. 6.2G). The resulting DEB model parameters can be found in Table 6.1.

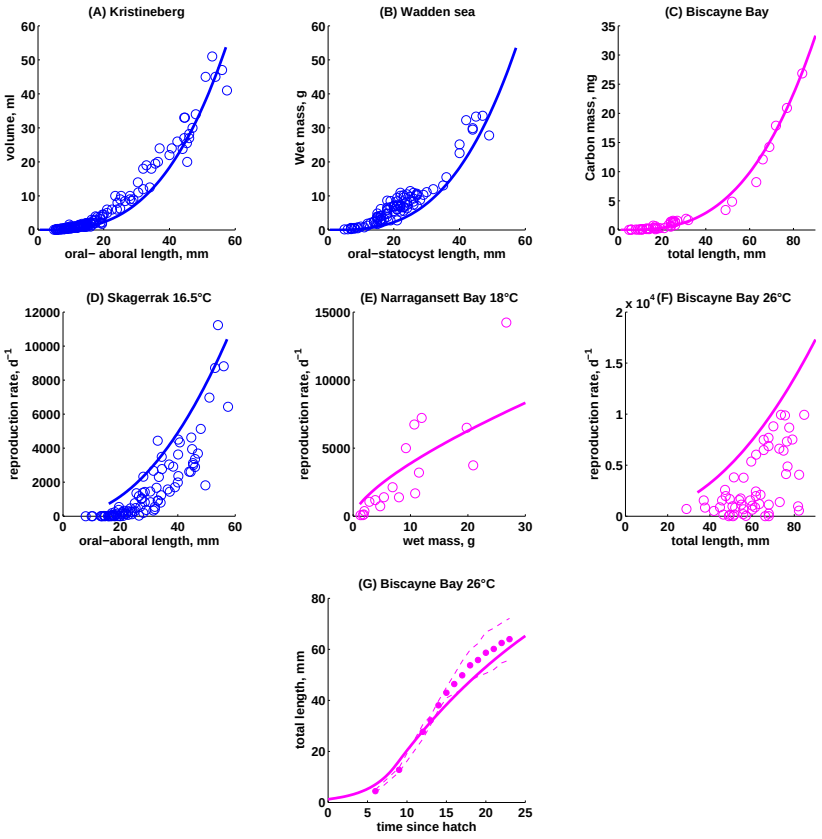

Figure 6.2: Uni-variate data used to estimate DEB model parameters. Symbols: data. Solid lines: DEB model predictions assuming $f = 1$. Top row (A–C): weight length relationships from Jaspers (2012) (A), Van Walraven *et al.* (2013) (B) and Reeve *et al.* (1989) (C). (D–F) reproduction rate length relationships from Jaspers (2012) (D), Kremer (1976a) (E) and Baker and Reeve (1974) (F). Finally we have the mean length against age in (G) from Baker and Reeve (1974), dashed lines represent the standard deviation around the mean values for the 6 individuals (circles).

Table 6.1: DEB model parameters. Parameters estimated from the data (Table 6.2 and Fig. 6.2A, B) at reference temperature T_{ref}. *denotes embryo values (multiply by ca. 8.6 to obtain adult values); † denotes values which are assumed, not estimated. OA: oral–aboral, OS: oral–statocyst, total: length including lobes.

Symbol	Value	Unit	Description
$\{\dot{p}_{Am}\}$	3.0*	J d^{-1} cm^{-2}	Maximum surface-area specific assimilation rate
K_X	0.8†	-	Digestion efficiency
K_X^P	0.1†	-	Faecation efficiency
K_R	0.95†	-	Reproduction efficiency
K	0.7	-	Allocation fraction to soma
$[\dot{p}_M]$	5.0	J d^{-1} cm^{-3}	Volume linked somatic maintenance costs
$[E_G]$	78.0	J cm^{-2}	Cost of synthesis of a unit of structure
$\dot{k}_J$	0.002†	d^{-1}	Maturity maintenance rate coefficient
$\dot{\nu}$	0.21*	cm d^{-1}	Energy conductance
Life stage parameters			
E_H^b	$1.5 \cdot 10^{-3}$	J	Cum. energy investment in maturation at birth
E_H^s	$4.4 \cdot 10^{-3}$	J	Cum. energy investment in maturation at the onset of metabolic acceleration
E_H^j	3.2	J	Cum. energy investment in maturation at metabolic metamorphosis
E_H^p	42.9	J	Cum. energy investment in maturation at puberty
Temperature parameters			
T_A	10,500	K	Arrhenius temperature
T_{ref}	293	K	Reference temperature
Auxiliary parameters			
$\delta_{\mathcal{M}}$	0.62 (OA and OS length)	-	Shape coefficient
$\delta_{\mathcal{M}}$	0.29 (total length)	-	Shape coefficient

Table 6.2: Observed and predicted values for zero-variate data used in the parameter estimation routine.

Datasets (units)	Observed	Predicted	T °C	Source
Age at birth (d)	1	1	18	Jaspers (2012)
Age at puberty (d)	13	14	26	Baker and Reeve (1974)
Oral–aboral length at birth (mm)	0.4	0.6		Jaspers *et al.* (2013)
Oral–aboral length at metabolic metamorphosis (mm)	6–10	7		Rapoza *et al.* (2005); Sullivan and Gifford (2004)
Total length at puberty (mm)	30	34		Reeve *et al.* (1989)
Organic matter content of an egg (µg)	0.25	0.24		Anninsky *et al.* (2007)
Ultimate wet mass (ml)	51	54		Raw data from Jaspers (2012)

In Sections 4.1–4.4 we will discuss insight gained and new questions raised through the analysis of literature data using DEB theory. The DEB model parameters in Table 6.1 reveal the link between energy allocation to different metabolic processes which have been computed for two relevant life-stages (Fig. 6.4) and key eco-physiological properties such as the organisms maximum storage capacity, the residence time of compounds in reserve and an intrinsic resistance to starvation. We will discuss this in Section 4.5 and we will further compare parameters obtained for *M. leidyi* with those of another holoplanktonic gelatinous plankton species *P. noctiluca*.

Metabolic acceleration and life history

We found that it was not possible to capture embryo development in combination with high adult reproduction rates without including metabolic acceleration. Type M metabolic acceleration occurs when incubation time is longer than expected based on adult values of $\dot{\nu}$ and $\{\dot{p}_{Am}\}$, length as function of age is upcurving and reserve density is not affected (Kooijman, 2014). We found that Type $\mathcal{M}$ metabolic acceleration is needed in the first place to capture embryonic development in combination with later juvenile and adult development, growth and reproduction. Furthermore, the embryo does not show developmental arrest and cleavage is continuous (pers. obs. C. Jaspers).

We compared the DEB model predictions with several published growth curves during the cydippid and transitional stages: Anninsky *et al.* (2007) (28 °C), Baker and Reeve (1974) (26 °C), Reeve and Baker (1975) (21, 26 and 31 °C), Stanlaw *et al.* (1981) (21 °C), Sullivan and Gifford (2007) (18–21 °C) and Jaspers (2012) (19 °C). Only the DEB model prediction for length against age from Baker and Reeve (1974), used for parameter estimation, is presented graphically in Fig. 6.2G.

The reason that we were only able to include the growth curve from one of the studies is that larval growth presents a variety of morphologies and it is not possible to fit the model to all assuming a same scaled functional response and a same maturity level for the onset of metabolic acceleration. The growth curve from Baker and Reeve (1974) was the only one describing growth over the full life-cycle where the individuals reached their maximum size (70–80 mm total length).

Part of the explanation for why larval growth seems to be different for every study might reside in the fact that the organism changes shape as well as diet during this time (Rapoza *et al.*, 2005; Sullivan and Gifford, 2004). However, our results

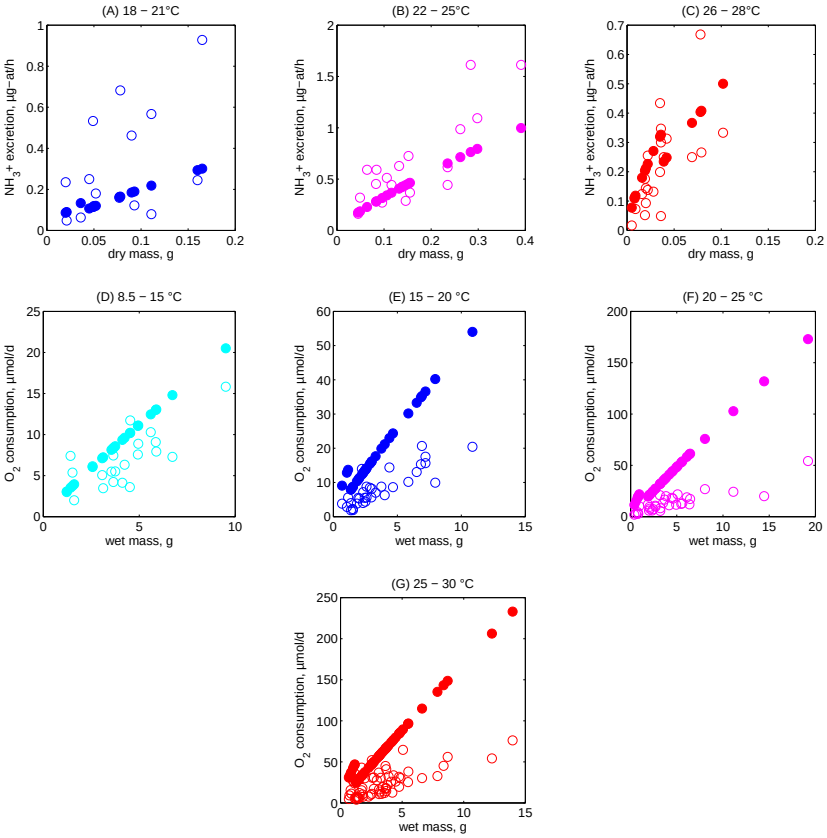

Figure 6.3: Predicted ammonia excretion and dioxygen consumption as function of dry (top row) or wet (bottom row) weight. Empty symbols: data from the literature. Full symbols: DEB model predictions assuming highest possible condition factor for each size (i.e. $f = 1$). Top row (A–C): data from Nemazie *et al.* (1993). The DEB model predictions account for contribution from assimilation to total ammonia excretion. Bottom row (D–G): data from Lilley *et al.* (2014). The DEB model prediction excludes contributions from assimilation to total dioxygen consumption.

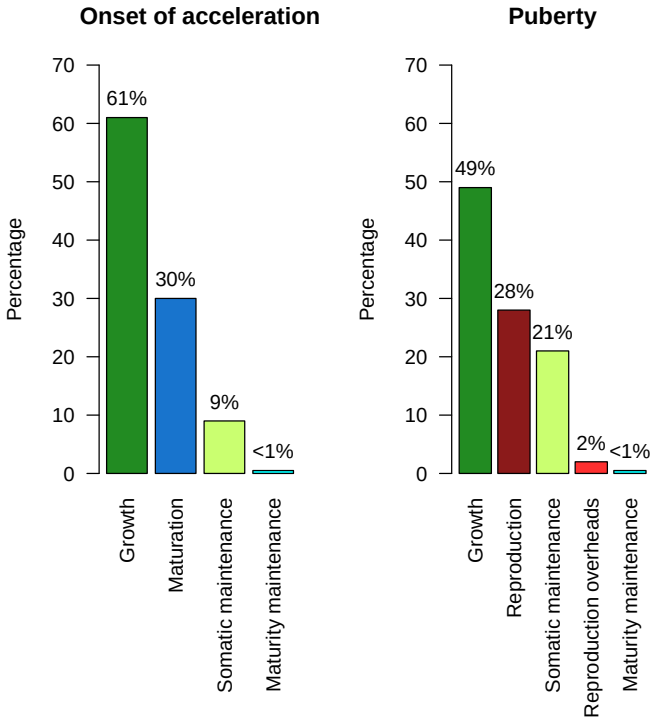

Figure 6.4: Percentage of energy mobilised from reserve towards each physiological process at the onset of metabolic acceleration (left) and at puberty (right).

also suggest that differences in timing of metabolic acceleration also contribute to these differences. This requires further research where growth is observed at different food levels and perhaps also for different feeding protocols. Overall, metabolism seems to accelerate at some point after birth and acceleration seems to stop before puberty. With the current parameter set (Table 6.1) the acceleration factor is about 8.6 and acceleration starts 2 days after birth at 0.7 mm OA length (f = 1, T = 20 °C).

In fact, this delay hints towards the possible metabolic flexibility for controlling generation time. Simulations show that at ad libitum food the metabolism starts to accelerate soon after birth and adult reproduction in the lobate stage can start as early as 2 weeks later at 26 °C This allows us to hypothesise that if food levels and temperature are low (say f = 0.3, T = 12 °C) then first reproduction after metamorphosis can be delayed for up to a year (van der Molen *et al.*, 2015). However, it has been shown that several ctenophore species, both cydippid and lobate, reproduce in the cydippid larval stage (Chun, 1892; Jaspers, 2012; Martindale, 1987) and it has been suggested by empirical data and modelling that this is a life history trait unique to ctenophores which enables populations to maintain themselves under high predation pressure (Jaspers, 2012). Further studies on larval population dynamics are necessary to investigate if animals can be trapped in the larval stage as suggested by DEB model and this parameter set or not.

Resource use and its acquisition are uncoupled in the DEB model because food is first assimilated into a reserve before being mobilised towards different metabolic processes. We compare the energy allocation of a small individual who just started accelerating against that of an individual at puberty in Fig. 6.4. The bar charts show what fraction of energy mobilised is allocated to each process as specified by the DEB model. From there we see that smaller individuals need to pay a factor 2 less maintenance relative to large individuals. This would be a highly ecologically relevant feature because small larvae can last a very long time at low food levels. One hypothesis is that the onset of metabolic acceleration might be triggered by an environmental factor and that delaying metabolic acceleration until food and temperature conditions are more favourable could help surviving periods without much food. A recent study on fish larvae suggests that this is the reason similar taxa of fish may or may not accelerate. The occurrence of metabolic acceleration was linked to the timing of spawning and whether or not conditions at hatch are favourable for growth (Lika *et al.*, 2014a).

The DEB model assumes an instantaneous switch from maturing to allocating to reproduction. However, in several studies, reproductive output of ctenophores has been shown to start at low rates after puberty (Baker and Reeve, 1974; Jaspers, 2012) and is hence poorly described by the DEB model. With the current parameter set in Table 6.1, the model predicts that full reproduction occurs 11 days after completed metamorphosis (f = 1, T = 20 °C). However, Jaspers (2012, Chap. 6) observed that the onset of slow reproduction coincided with the completed metamorphosis of *M. leidyi* from transitional to fully lobate animals, while both Reeve and Baker (1975) and Reeve *et al.* (1989) reported that first reproduction occurs later than the finished metamorphosis to adult lobate stage. Thus it is still hard to ascertain how morpho-anatomical transformations relate to puberty as

defined by the DEB model and perhaps this even differs between American and European populations.

The initial slow start of reproduction might also relate to investing in the male function. At puberty, investment into reproduction represents 28 % of the energy mobilised from reserve while overheads linked to gamete formation comprise 2 % of the total amount mobilised (Fig. 6.4). It is still unclear what fraction of the energy budget is invested into the male function. We interpret the above described discrepancies between model and data as evidence that it might represent more than the 5 % reproduction overheads. A recent DEB modelling study on the simultaneous hermaphrodite *Limnea stagnalis* suggests that investment into the male function might represent up to 50 % of the energy allocated towards reproduction (Zimmer *et al.*, 2014). The authors also show that the investment can be modulated by environmental factors.

Reproduction rates and weight–length relationships

In Fig. 6.2A–C we present DEB model predictions for three weight–size relationships: volume against OA length, WM against OS length and carbon mass against total length. Overall the predictions are in the ballpark of the observed values although predictions for intermediate size classes are closer to the lower values observed.

One interpretation of the shape coefficient is that $L/\delta_{\mathcal{M}} = L/L_w$. Thus, increasing $L/\delta_{\mathcal{M}}$ results in increasing structural length relative to physical length; decreasing $L/\delta_{\mathcal{M}}$ results in increasing the physical length relative to structural length. During parameter estimation we used a constant value for $L/\delta_{\mathcal{M}}$; it is very likely that $L/\delta_{\mathcal{M}}$ changes over ontogeny. Indeed, shape does change over ontogeny and a same shape coefficient might not apply for cydippid and transitional juveniles as for lobate adults. If we present cumulative number of eggs and length against time since hatch for the 6 individual ctenophores from Baker and Reeve (1974, Table 2), then the individual who reached the largest size does not have the highest cumulative egg production (not shown). This could relate to a distortion of the length measurement which might no longer be proportional to structure.

It is also likely that reserves contribute somehow to total length since lobes shrink first during starvation (pers. obs. C. Jaspers, unpublished van Walraven 2014) . The predictions for carbon and nitrogen weights against OA length from Sullivan and Gifford (2004) match observed values up to 6 mm, but predictions exceed the observed values after that (not shown). However the organism changes shape around that size and we did not adjust $L/\delta_{\mathcal{M}}$ for changes in shape within a study.

Model predictions for reproduction rate at 16.5 °C are consistent with the highest values for 30–60 mm OA length individuals (Fig. 6.2D). However predictions exceed the observed values for individuals less than 30 mm. The predicted reproduction rates for a given wet mass at 18 °C fall midway between the observed higher and lower boundaries (Fig. 6.2E). The DEB model over predicts reproduction rates against total length reported in Baker and Reeve (1974) (Fig. 6.2F). However the temperature in the field was not reported and we assumed that it was 26 °C. The

reproduction rate against length data from Jaspers *et al.* (2011) taken at 12.5 °C (Kattegat, October 2009) suggest that the performance of those individuals differs notably from that of the first two datasets, but this is discussed in more detail in the following subsection.

Thermal tolerance

In order to compare the value of the Arrhenius temperature obtained in this study with Q_{10} values provided in the literature on *Mnemiopsis*we can make use of the following relationship: $Q_{10} = \exp\left(\frac{10\,T_A}{T\,(T+10)}\right)$ (Kooijman, 2010). Thus with T_A = 10.5 kK, the Q_{10} is 3.5, 3.3 and 3.1 at 12, 18 and 26 °C respectively. This is within the range of values reported by Kremer (1977) and *M. leidyi* does have a very broad temperature tolerance of 2 till over 30 °C (Costello *et al.*, 2006; Purcell *et al.*, 2001). However what the optimal temperature range and how metabolic rates are affected by temperature outside of that range is unclear at the moment. The simple Arrhenius relationship only applies within the thermal tolerance range. Above or below the upper and lower boundary physiological performance starts to deviate from that predicted by the relationship, the idea being that the organism is somehow stressed.

The DEB model predictions for reproduction rates against size at 16.5 and 18 °C are within the observed range (Fig. 6.2D–E). However the reproduction rates of individuals caught in the Kattegat at 12.5 °C (Jaspers *et al.*, 2011) cannot be captured with this TA (not shown). A TA of about 25 kK as opposed to 10.5 kK reported in Table 6.1 would be required to capture differences between reproduction rates between studies. Such a high TA does not fit well with a lot of the other datasets and we do not know of studies on other organisms where such a high value has been recorded.

In short, the current results suggest that 12.5 °C might already be below the optimal temperature window. This highlights the need to perform more detailed physiological experiments to assess the optimum temperature tolerance window. The current modelling framework will help in this endeavour because it will be important to take into account the combined effects of both food and temperature on physiological rates. We further detail why in the following subsection.

subsection*Respiration and excretion rates The respiration and excretion rates were not included in the parameter estimation since values across studies showed some discrepancies as will be discussed herein. Using the parameters (Table 6.1) estimated simultaneously from data presented in Table 6.2 and Fig. 6.2 we computed predictions for ammonia excretion at three different temperature classes from Nemazie *et al.* (1993) (Fig. 6.3A–C) as well as dioxygen consumption at 4 temperature classes from Lilley *et al.* (2014) (Fig. 6.3D–G). In line with the original protocol we excluded contributions from assimilation from the predictions for data from Lilley *et al.* (2014), Kremer (1976a) and Kremer (1982). The model predictions are in line with ammonia excretion as function of dry mass from Nemazie *et al.* (1993). However the model overestimates the dioxygen consumption measured in Lilley *et al.* (2014) for organisms above 3 g (Fig. 6.3D–G). Of importance to note

is: the higher the temperature, the higher the discrepancy. The DEB model also over-predicts carbon dioxide, ammonia and dioxygen excretion and consumption rates reported in Kremer (1976b, Appendix) and in Kremer (1982) (not shown).

Nemazie *et al.* (1993) suggest that the differences between their values and that recorded in Kremer (1976a) stem from differences in contribution of organic matter to total dry mass which in turn are the result of differences in ambient salinity between studies. Nemazie *et al.* (1993) worked at an ambient salinity of 6–12 while Kremer and coworkers performed studies on organisms near the open ocean and Key Biscayne so the ambient salinity was probably closer to 35 (pers. comm. P. Kremer). But these difference might also stem from the fact that the metabolism of large size classes is very sensitive to short term fasting relative to that of the small size classes and that the problem is more severe the higher the temperature. The reason that larger sizes classes suffer more from food shortage is that a higher percentage (relative to a smaller individual) of energy mobilised from reserve must be allocated to cover somatic maintenance costs (see Fig. 6.4).

Thus at high temperatures and when measuring metabolic rates on large individuals it would probably be hard to detect the upper thermal tolerance limit without including both effects of food and temperature on physiological rates in a mechanistic way as done in this study. This might also explain why the DEB model systematically over estimates respiration and excretion rates for many of the studies while it does not for Nemazie *et al.* (1993) (who only worked with small size classes).

We must bear in mind that the model assumes no effect of temporary fasting on the rate of reserve mobilisation prior to the measurements (Section 3.5). There is some empirical support that short term starvation does affect the slope of dioxygen consumption over time in *Oithona davisae* where the dioxygen consumption rate is first fast then slow over the experimental period for starved nauplii (Almeda *et al.*, 2011, Fig. 2B).

Inter-species comparison of parameter values Of interest to note here is that the different parameters each have a specific physiological interpretation. Patterns in values for hundreds of animal species are being studied across taxa to try and ultimately link eco-physiological properties to specific combinations of parameters values (Lika *et al.*, 2014b). Parameters for the standard DEB model for all of these animal species can be found in the online library of DEB model parameters: the Add_my_Pet collection, `http://www.bio.vu.nl/thb/deb/deblab/add_my_pet/Species.html`. The model captures quite a few aspects of the individual's energy budget very realistically. The comparison between *M. leidyi* and *P. noctiluca* is interesting in that both are holoplanktonic gelatinous organisms.

M. leidyi has a specific density of 0.003 g cm^{-1} while that of *P. noctiluca* is around 0.01 g cm^{-1} (Augustine *et al.*, 2014b). Thus there is a factor 3 more water in the former species relative to the latter. After accounting for differences in water content the comparison indicates a rather severe metabolic difference: *M. leidyi* seems to be comprised of 80 % structure while *P. noctiluca* is comprised of 98 % reserve. Volume-linked somatic maintenance costs of 5 J cm^{-3} d^{-1} might seem low compared 164 J cm^{-3} d^{-1} for *P. noctiluca* or even to the proposed 'typical' 18 J cm^{-3} d^{-1} after Lika *et al.* (2011). But in order to compare, $[\dot{p}_M]$ should be

corrected for the water content and $[\dot{p}_M]/d_V \approx 1.7$ kJ g^{-1} d^{-1}. *P. noctiluca* was found to invest 15 kJ g^{-1} d^{-1} (Augustine *et al.*, 2014a), and zebrafish 2.5 kJ g^{-1} d^{-1} (Augustine *et al.*, 2011). While the 'typical' value for a generalised animal with d_V = 0.1 would be 0.2 kJ g^{-1} d^{-1}. Thus *M. leidyi* might waste assimilates to boost metabolism and shorten generation time as suggested by the 'Waste to Hurry hypothesis' (Kooijman, 2013).

The energy conductance of 0.21 cm d^{-1} before acceleration and 1.8 cm d^{-1} afterwards for *M. leidyi* is very high compared to most of the values listed in the Add_my_Pet library. $\dot{\nu}$ plays an important role in embryo development but also in shaping the relationship of O^2 consumption or NH^3 as function of length and weight. The physiological interpretation is that the organism has a high mobilisation rate of its reserves and so if we come back to the previous subsection it means that the time it takes for mobilisation to be affected by fasting will be short relative to species with a lower $\dot{\nu}$, all else the same. The high $\dot{\nu}$ entails a low maximum reserve capacity of about 15 J cm- 3 or 5 kJ g- 1. In comparison *P. noctiluca* has 14 kJ cm- 3 or 1400 kJ g- 1. A rough estimate of time till starvation can be derived by $[E_m]/[\dot{p}_M] \approx 3$ whereas *P. noctiluca* has 84 days.

Conclusions

In this section we highlight the three most important contributions of this study to the literature on *M. leidyi*'s ecophysiology. First, *M. leidyi* has a large fraction of structure relative to its reserve. We estimate somatic maintenance costs to be 5 J d^{-1} cm^{-3}. This is lower than many of the values found for other animal species (Lika *et al.*, 2011). However once we account for the very high water content in this species, maintenance costs per gram of water free structure are actually higher than many of those species.

The high somatic maintenance in combination with the high energy conductance has profound consequences for the shape of e.g. respiration rate against size (length or mass) relationships. A day of starvation for a 1 g and a 10 g wet weight individual is not equivalent in terms of how it will affect metabolic rates. The low reserve capacity means that *M. leidyi* shrinks, i.e. reduces its amount of structure in response to prolonged starvation.

Second, physiological rates might already start deviating from the Arrhenius relationship around 12 °C. A constant Q_{10} should not be used for comparing rates spanning large temperature ranges. The problem of effects of temporary starvation on the large size classes is exacerbated with increasing temperatures making it hard to detect the upper temperature range at which rates start deviating from the Arrhenius relationship. Third, it is likely that something triggers the onset of metabolic acceleration for newly hatched individuals. Until we know more about this, experimentalists should be aware that results from experiments might differ a lot depending on whether or not the experiment starts directly after hatch or sometime after hatch when the organism is metabolically accelerating. More dedicated study on this species intrinsic phenotypic plasticity is needed in order to assess effects of genetic differences between the different populations in European

and the USA. Our results suggest that part of the diversity in larval growth as well as size and timing of first reproduction may stem from the delay in starting metabolic acceleration in addition to the already reported insight that dietary requirements change.

A reconstruction of the dynamics of size structure over time observed in the field in combination with the knowledge herein would be very helpful to get some idea about the condition of the individuals in the different seasons. Together with new ideas presented here on the slow initial development we might get some understanding of just how long they remain small and then how in a matter of weeks they can grow to more consequent sizes (40– 60 mm OA length) and sustain high reproduction rates. The observed large variation in size classes of *M. leidyi* in the field (e.g. Haraldsson *et al.*, 2013, Fig. 7) might relate to environmental factors that affect the delay in metabolic acceleration.

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Chapter 7

Ctenophores invading Amsterdam: Differential response to different salinity levels in two invasive *Mnemiopsis leidyi* populations

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Abstract

One of the most notorious invaders in the marine environment is the ctenophore *Mnemiopsis leidyi*, originating from the American East coast and well known for its invasion in the Black Sea in the 1980s. Although the species can tolerate a broad range of temperatures and salinities in its native range, low salinity limits the range expansion of *M. leidyi* in parts of northern Europe. Recently, large *M. leidyi* blooms have been observed in the brackish North Sea Canal near Amsterdam in the Netherlands, where fish are sometimes completely clogged with the ctenophores.

To investigate whether tolerance for low salinity levels is different in this area, a common garden experiment was designed in which the phenotypical response of the low salinity Amsterdam population was compared to a nearby population, originating from the higher saline Wadden Sea. Individuals from both locations were induced to spawn and half of the spawn was grown and acclimatised to low salinity (S = 8) while the other group was acclimatised to high salinity (S = 33) conditions. The offspring of these four groups, hence the F2 generation, was used in a 32 day experiment during which survival, growth and reproduction were measured.

Genotyping of the animals surviving at the end of the experiment revealed high differentiation between sub-populations of origin. Within the Amsterdam sub-population high genetic differentiation was found.

This experiment shows that *M. leidyi* can complete its entire life cycle at a salinity of 8. However, low salinity treatments showed overall high mortality of 53 and 83 % for Amsterdam and Texel populations, respectively. *M. leidyi* originating from the Amsterdam population showed highest reproduction rates and earlier maturation at low salinity treatments compared to all other groups. This suggests that *M. leidyi* can sustain populations at salinities as low as 8. More research is needed to find out whether this result is based on selection for high performance genotypes at low salinities or adaptation. The presence of a low salinity tolerant genotype which is significantly different from the current North Sea genotype suggests that *M. leidyi* can thrive in areas where salinity was thought to be limiting its reproductive capacity. Re-current invasions of genotypes with different environmental tolerances could cause an unexpected spread of animals in habitats for which environmental parameters were previously thought to be limiting.

Introduction

Invasive species are characterized by surviving, reproducing and spreading after their introduction in a new environment (Elton, 1958; Mack *et al.*, 2000). Although this phenomenon is not new, the amount of species introductions and succesful invasion has increased significantly in the past decades (Di Castri, 1989). This trend is likely to continue due to globalization and has been suggested to lead to a homogenization of the worlds biota (Lodge, 1993; McNeely, 2006). Once invasive species are established in the new environment, they have the potential to change the ecological properties of a system, such as nutrient cycling or species interaction (Vitousek, 1996), and thereby altering ecosystem functioning (Bax *et al.*, 2003).

An example of an invasive species with documented ecological consequences is the holoplanktonic, lobate ctenophore *Mnemiopsis leidyi* A. Agassiz 1865, native to the eastern coast of the American continents (Kideys, 2002). It is characterised by high growth, feeding rates and high reproduction rates (Stanlaw *et al.*, 1981; Reeve *et al.*, 1989; Colin *et al.*, 2010; Jaspers *et al.*, 2015) and egg production has been shown to depend on size, food availability, water temperature and salinity (Kremer, 1976b; Reeve *et al.*, 1989; Jaspers *et al.*, 2011; Gambill *et al.*, 2015).

Mnemiopsis leidyi can survive in a wide range of environmental conditions; the species has been found in salinities between 2–47 (Marambio *et al.*, 2013; Purcell *et al.*, 2001) and water temperatures ranging from less than 1 °C (Costello *et al.*, 2006) to 32 °C (Purcell *et al.*, 2001). However, egg production is very low at a salinity of 6 in both field collected (Costello *et al.*, 2006) and lab-grown (Jaspers *et al.*, 2011) ctenophores.

The first introduction of *Mnemiopsis leidyi* was in the Black Sea in the 1980s (for review see Purcell *et al.*, 2001), where it contributed to a collapse of planktivorous fish populations (Bilio and Niermann, 2004) . However, this collapse in fisheries was mainly caused by a combination of eutrophication and overfishing as opposed to predation of *M. leidyi* on fish recruits (Daskalov, 2002; Daskalov *et al.*, 2007; Bilio and Niermann, 2004). This collapse had a substantial impact on fisheries in the area (Knowler, 2005).

From the Black Sea, the species spread out towards the Caspian, Aegean and Mediterranean Seas (Costello *et al.*, 2012), while a second independent invasion from the north east coast of the USA (Reusch *et al.*, 2010; Ghabooli *et al.*, 2011) resulted in blooms in the North Sea, Baltic Sea and adjacent waters following 2005 (Faasse and Bayha, 2006; Riisgård *et al.*, 2007, 2010; Oliveira, 2007).

In coastal waters of the Netherlands, *Mnemiopsis leidyi* has been found in the western Wadden Sea (Van Walraven *et al.*, 2013), Eastern Scheldt, saline Lake Grevelingen (van Walraven et al. in prep), but also in the low saline North Sea Canal in Amsterdam. Large blooms of *Mnemiopsis leidyi* have been observed in the North Sea Canal in summer since 2006, with clogging of fishing nets causing problems on a yearly basis (P. Ruijter and M. Melchers pers. comm.). The North Sea Canal is a 21 km long shipping canal, constructed in 1876 to enable ships to reach the Amsterdam port directly from the North Sea. It runs from the North Sea coast at IJmuiden towards the IJ bay in Amsterdam, which is connected to the freshwater Amsterdam Rhine Canal. Due to fresh water inflow from the east and salt water

inflow from the west, the water is brackish and highly stratified year-round (van Haaren and Tempelman, 2006). The area is known for its amount of invasive species, including the North American amphipod *Melita nitida* (Wijnhoven and Hummel, 2009), the red-gilled mud worm *Marenzelleria neglecta* (van Moorsel *et al.*, 2010) and several molluscs such as the Asian clam *Corbicula fluminea* and the Pacific oyster *Crassostrea gigas* (van Haaren and Tempelman, 2006).

The surface salinities found at the sampling sites (2–8) appear too low for sufficient reproduction of *Mnemiopsis leidyi* to maintain a population since field- and experimental studies demonstrated that salinity restricts the range expansion of *M. leidyi* into low salinity (<10) regions of the Baltic Sea (Jaspers *et al.*, 2011; Lehtiniemi *et al.*, 2012; Jaspers *et al.*, 2013). That raises the question whether the population in Amsterdam is originating from the population in the North Sea, or that it somehow differs.

At present there are several hypotheses to explain this unexpected high abundance of *M. leidyi* in the low saline North Sea Canal:

1. The Amsterdam population has adapted to a low saline environment;
2. The Amsterdam population has not adapted but reproduces and survives in deeper waters waters of the stratified North Sea Canal where the salinity is higher;
3. The Amsterdam population solely exists of individuals flushed into the canal from the North Sea;
4. The Amsterdam population is not originating from the North Sea population, but from another population witha different phenotypical response to low salinities.

If hypothesis 1 is true, the Amsterdam population has a different phenotype and genotype than the population in the North Sea under the same salinity regime. This would be caused by population differentiation, and would show that *M. leidyi* is even more adaptive than suggested before. If hypotheses 2 or 3 are true, the Amsterdam population has the same physiological response to salinity as the North Sea population. If hypothesis 4 is true, the Amsterdam population has a different functional response to environmental conditions than a North Sea population, which is based on genetic differences. As the port of Amsterdam handles about 7000 ships annually (Port of Amsterdam, 2013), propagule pressure could be very high due to the frequent ballast water discharges.

In this paper we test these hypotheses to investigate whether population differentiation can explain the high numbers of *M. leidyi* near Amsterdam. Common garden experiments are used extensively to differentiate between phenotypic plasticity and genotypic population differentiation (Bossdorf *et al.*, 2005). In this study, a common garden experiment was designed in which the survival, growth and reproduction of two *M. leidyi* populations originating from two environments with different salinity levels were compared. Individuals from both locations were kept on both a low (8) and a high (33) salinity, spawned, and followed for 32 days. Genetic variation among and within experimental treatments and locations was

investigated at the end of the experiment by genotyping survivors (Reusch *et al.*, 2010).

Material and methods

Specimen collection

Ctenophores were collected in the North Sea Canal area (hereafter called 'Amsterdam population'), Amsterdam port, in the Jan van Riebeeckhaven at 52.4164 N, 4.8410 E, on 27-08-2013 and in the NIOZ harbor on Texel (hereafter called 'Texel population') at 53.0058 N, 4.7966 E, on 28-08-2013 (Fig. 7.1) which lies at the westernmost entrance of the Wadden Sea from the North Sea. These areas represent a low and a high salinity area with *in situ* salinities during collection of 8 and 33 for Amsterdam port and the Wadden Sea, respectively. Animals were collected by dipping plastic jars in the surface layer. They were shipped on 28-08-2013 to the Sven Loven Institute of Marine Sciences in Kristineberg, Sweden, in 1 L PVC Kautex jars, where they arrived the next day. First, they were acclimatised at natural light conditions and in situ salinity levels for one week. All low saline water was produced by diluting 0.2 μm filtered sea water with demineralised water. During acclimatisation animals were fed 3-week old cultured *Acartia tonsa* copepods at a concentration of approximately 40 copepods L^{-1}.

For all experiments *Acartia tonsa* copepods and nauplii were used as food, cultured on a diet of *Rhodomonas salina* and *Thalassiosira weissflogii*. Salinity will not only affect the ctenophores studied, but also the copepod species used as food source. The *A. tonsa* copepods for this experiment were all cultured at a salinity of 33. *A. tonsa* is very resilient to sudden salinity changes; in an experiment conducted using copepods from the same culture as the one used in this experiment, mortality was only 3 % when the copepods were introduced to S = 8 water from S = 32 (Calliari *et al.*, 2008).

Adaptation

To exclude any phenotypic changes occurring during acclimatisation to different salinities in the experiment, ctenophores needed to be grown from egg to adult in the same salinity. Therefore, field caught animals were kept in the lab for 1 week before they were induced to spawn at their natural salinity range. The resulting larvae were acclimatized to the different salinity levels with one of the groups kept on its original salinity (8 or 33), while the other group was slowly acclimatized to the other salinity level over 16 days with a 3 unit change at S >14 and a 1 unit change at S <15. During the daily water changes the animals were measured under a binocular microscope and egg production was checked by reverse filtrating the entire container using a funnel with a 60 μm mesh size filter, and counting the remaining eggs (data not shown).

Figure 7.1: Map showing the two sampling locations of this study. a. overview, b. Texel detail, c. North Sea canal/Amsterdam detail. Blue = sea, white = inshore waters.

Experimental design

After the F1 generation started to produce sufficient eggs, the adults were removed and the F2 larvae were grown at a food concentration of 1900 L^{-1} 2-day old *A. tonsa* nauplii, similar to the initial food concentration in the experiment, for four days to prevent initial larval mortality caused by handling. Following this, larvae were collected and used in a common garden experiment. Each treatment had 4 replicates with 15 individuals per container. The experiment was carried out in a 19 °C climatised room. Containers were kept in the dark to ensure an even copepod food concentration in the water column. Aeration was not used.

Every day all individuals per group were measured using a binocular microscope. Animals smaller than 1 mm were measured in a droplet of water and animals larger than 1 mm were measured in a petri dish. After sizing, all animals were transferred to a new container. Initial food concentrations were 1900 L^{-1} 2-day old nauplii for containers with ctenophores smaller than 1.8 mm as young *M. leidyi* larvae are delicate and easily harmed by adult copepods (Stanlaw *et al.*, 1981), and 40 L^{-1} 3-week old copepods for containers with individuals larger than 1.8 mm. These values were chosen to obtain a start food concentration of 100 μg C L^{-1} to ensure

maximum growth and egg production (Jaspers *et al.*, 2015). Ctenophore density and food availability where kept constant by adjusting the container volume based on equation 7.1:

$$V = \frac{antL^b}{\ln \frac{C_0}{C_t}} \tag{7.1}$$

where V is the water volume, n the amount of individuals in a container, t the time in the container (21 hours, as the measuring and transferring took 3 hours), L the average oral-aboral length of the individuals, C_0 the initial food concentration (1900 L^{-1} for nauplii, 40 L^{-1} for copepods), and C_t the food concentration at the end of the time. The maximum food concentration reduction was allowed to be 30 %, so C_t was 1300 L^{-1} and 21 L^{-1} respectively. The parameters a and b were calibrated by previous clearance rate experiments using different *M. leidyi* size classes (Friis-Møller, unpublished results). The water volumes used differed between 0.25 L at the start of the experiment, and 100 L at the end of the experiment. Animals were transferred to new containers every day. From day 5 to day 22 after hatch, ingestion rate was determined by counting two sub-samples while after 21 days- additionally the entire water volume was filtered via reverse filtration and all ctenophore eggs were counted.

Survival was measured by counting the surviving individuals on a daily basis. Growth was assessed by measuring the change in oral-aboral (OA) and total length (TL) over time. In this case the oral-aboral length of a ctenophore is the length from the mouth up to the top, and the total length is including the lobes. The TL/OA ratio was used to assess the developmental stage of the animals with a ratio of 1 = larvae, 1.5 = transitional and 2 = adult.

For analysis of carbon and nitrogen content animals remaining at the end of the experiment were frozen at -20 °C and freeze dried for at least 24 hours until constant weight. Carbon and nitrogen content were measured on a Flash 2000 Organic Element Analyzer at the Royal Netherlands Institute for Sea Research, Texel, Netherlands.

Analysis

Data exploration was performed as outlined by Zuur *et al.* (2010). Statistical analyses were performed using R 3.1.2 (R Core Team, 2014).

Differences in final length between the groups were analyzed with a linear mixed effect model using the nlme package (Pinheiro and Bates, 2000), in which the length of a ctenophore in container k from location i and salinity j is represented by the model:

$$Length_{ijk} = \alpha + salinity_i + a_k + \epsilon_{ijk} \tag{7.2}$$

Because measurements of individuals within the same container are not independent, container k was included as a random factor:

$$\alpha_k \sim N(0, \sigma_a^2) \tag{7.3}$$

Due to the fact that the variance between groups was different, a weight factor was added which allowed each combination of location and salinity to have its own

variance (Zuur *et al.*, 2009):

$$\epsilon_{ijk} \sim N(0, \sigma^2_{ij}) \tag{7.4}$$

Restricted Maximum Likelihood (REML) was used for parameter estimation.

Cumulative egg production was calculated as the sum of the average egg production per individual per day. Differences in egg production between treatments and sub-populations were investigated with a negative binomial generalized linear model (GLM), using the MASS package (Venables and Ripley, 2002). The explanatory variables for this model were location and salinity with model selection based on Akaike Information Criterion (AIC) values. Location-treatment combinations are given a name based on their location of origin (Texel or Amsterdam) and the salinity level (33 or 8).

Genetic analysis

At the end of the experiment, seven species-specific, highly polymorphic microsatellite loci (Reusch *et al.*, 2010) were used to genotype surviving animals from all four experimental groups (n = 38 for Texel 33, 14 for Texel 8, 12 for Amsterdam 33 and 14 for Amsterdam 8). Individuals were frozen in 20 mL glass tubes at -80 °C and freeze dried for 24h at -51 °C. For molecular analyses, tissue was lysated in 100 µL low TE buffer (10 mM Tris, 0.1 mM EDTA) for 1 h at 56 °C, while shaking and 1 µl extract used as template for the PCR reaction. Primer concentrations and PCR conditions followed published protocols (Reusch *et al.*, 2010; Bolte *et al.*, 2013).

Amplified fragments were analyzed on an ABI 3130 genetic analyzer using Rox-350 (Applied Biosystems) as internal size standard. Allele sizes were scored using the software GENEMARKER v1.91 (SoftGenetics, LLC). Tests of Hardy-Weinberg (HWE) and linkage disequilibrium (LD) were performed using ARLEQUIN v3.5.1.2 (Excoffier and Lischer, 2010) and results adjusted for type I error (Narum, 2006) following the Benjamini-Hochberg False Discovery Rate (B-H FDR) procedure (Benjamini and Yekutieli, 2001). Pairwise F_{ST} values were computed in ARLEQUIN v3.5.1.2, using non-parametric permutation procedures. All tests were performed with an initial alpha level of 0.05 and corrected following the B-H FDR method (Benjamini and Yekutieli, 2001).

Distribution of molecular variance amongst groups was tested using an analysis of molecular variance (AMOVA, ARLEQUIN v3.5.1.2). Two sets of AMOVAs were performed with location as primary hierarchical grouping and salinity as nested factor in the first analyses and salinity as primary hierarchical grouping and location as nested factor in the second analyses.

The software STRUCTURE v2.3.4 (Pritchard *et al.*, 2000) was used to infer genetic clustering without a priori assumption about expected number of clusters. STRUCTURE implements a Bayesian inference algorithm for detecting the number of clusters (k) that best explain genetic variation within a multilocus dataset. We used 100,000 reiterations for the burn-in and 1,000,000 Markov Chain Monte Carlo (MCMC) repetitions. Probabilities were calculated for k ranging from 1 to 5 with five replicates for each k and the most likely number of k's inferred through the Evanno method (Evanno *et al.*, 2005) using the internet interphase STRUCTURE

HARVESTER (Earl *et al.*, 2012). After all data were analyzed, we repeated this procedure analyzing Texel and Amsterdam sub-populations separately to detect possible sub-structures (Pritcharda *et al.*, 2009).

Results

All ctenophores of the F1 generation that were included in the acclimatization treatments survived, except one individual of Amsterdam origin. Ctenophores from the Amsterdam group were smaller, especially in the group that was kept at a constant low salinity of 8 (Fig. 7.2). Egg production was first observed on day 10 and was highest in the Amsterdam group kept at a constant low salinity (Fig. 7.3).

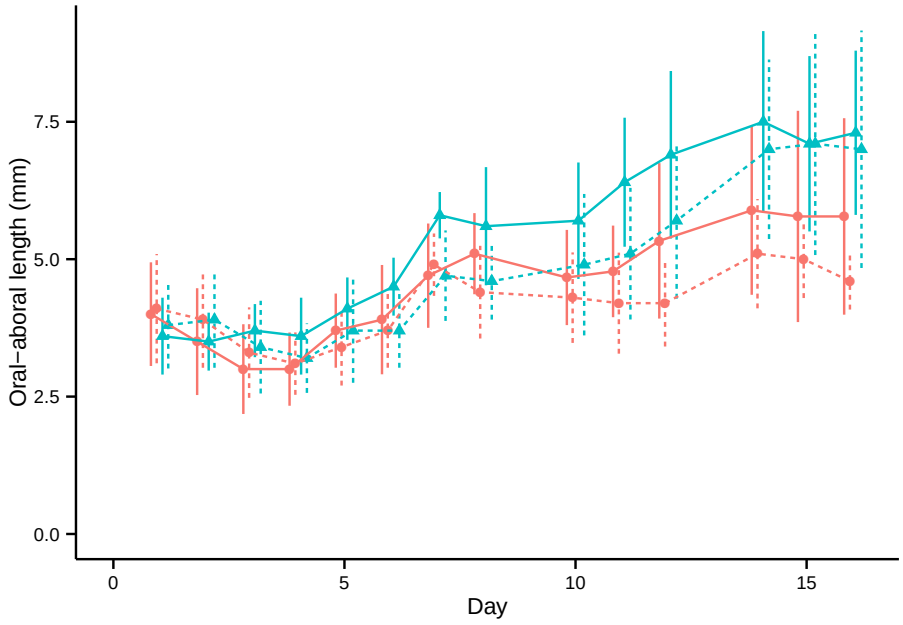


Figure 7.2: Mean Oral-Aboral (OA) length with standard errors for each container during acclimatisation. Red lines and circles: Ctenophores collected in Amsterdam, Blue lines and and triangles: ctenophores collected on Texel. Solid line: salinity acclimatisation, dashed line: constant salinity. Points are slightly offset to show the overlapping standard errors.

Experiment

Mortality

During the common garden experiment mortality was very high for the low salinity groups, especially in the initial five days of the experiment, from an age of 4–9

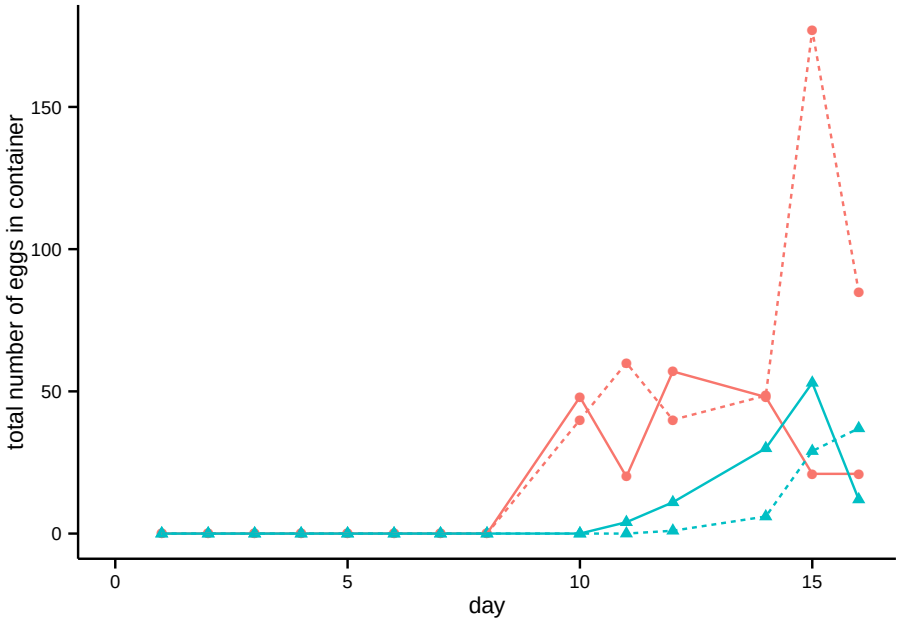


Figure 7.3: Total number of eggs counted per day for each container during acclimatisation (n=10 ctenophores in all containers except in the Amsterdam acclimatisation treatment where one individual died). Red lines and circles: Ctenophores collected in Amsterdam, Blue lines and and triangles: ctenophores collected on Texel. Solid line: salinity acclimatisation, dashed line: constant salinity.

days (Fig. 7.4). In some replicates over 60 % of larvae died on the first day. To maintain an approximately equal number of animals per replicate container, the two replicates with the highest mortality for both Amsterdam and Texel at low salinity were combined on day 7 so that at the end of the experiment there were 3 replicates per group for the low salinity treatments and 4 for the high salinity treatments. During the first six days 68–73 % of the ctenophores in the low salinity groups and 17–47 % of the ctenophores in the high salinity groups died. Mortality remained low after the animals reached an age of approximately ten days, except in the Amsterdam-collected group at high salinity, where mortality was high throughout the experiment. Overall mortality was 73–80 % in Amsterdam 8, 40–73 % in Amsterdam 33, 53–83 % in Texel 8 and 13–53 % in Texel 33.

Growth

The growth of the four groups showed distinct patterns (Fig. 7.5). For the groups cultured on the low salinity, growth was initially reduced but gradually increased later in the experiment. Compared to the high salinity groups, lobe forming was delayed in these groups. The Amsterdam high salinity group reduced in length

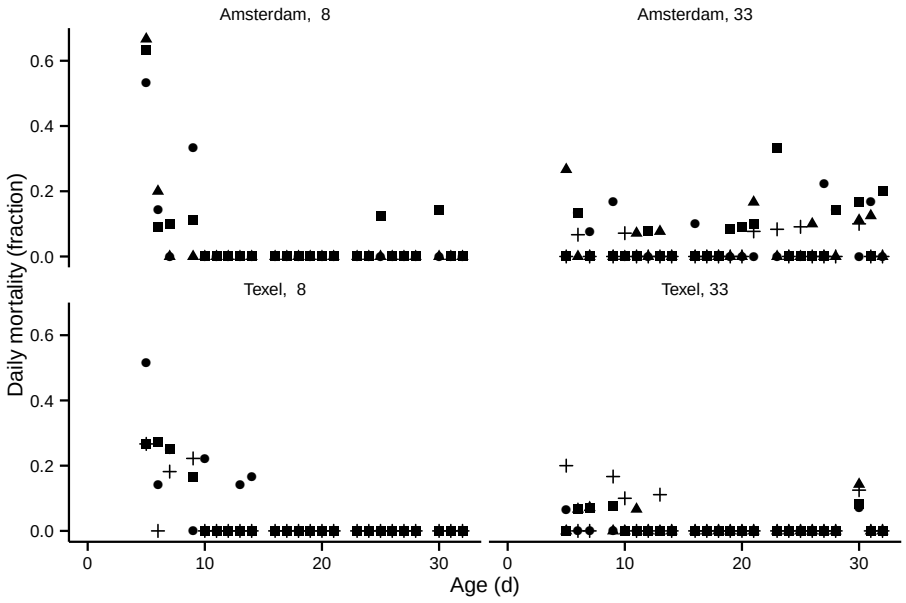


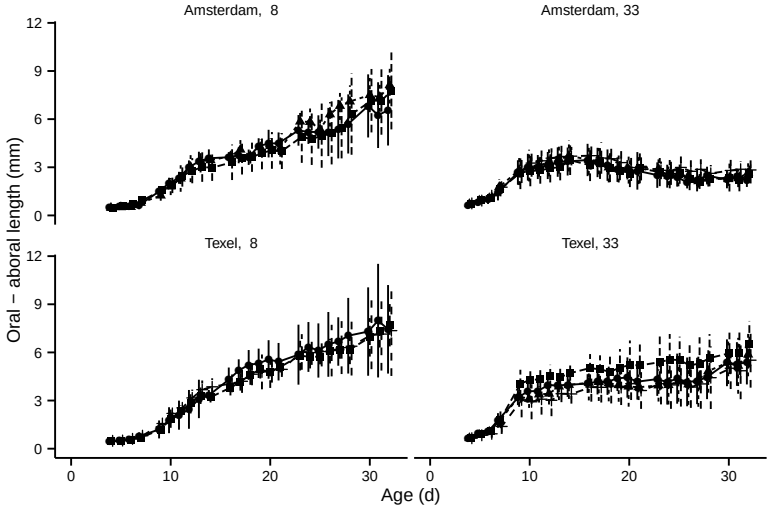
Figure 7.4: Daily mortality for each container in the experiment. The experiment started with 4 replicates per treatment (n = 15 ctenophores per container) but due to very high mortality in the containers of the low salinity treatment of both populations the two replicates with the highest mortality for both Amsterdam and Texel at low salinity were combined on day 7 to maintain an approximately equal number of animals per container. Different symbols represent the different replicates.

after fifteen days; this was observed in all replicates. At the end of the experiment, the variety in length differed per group (Table 7.1). On the last day, the oral-aboral length was different for each location and every salinity (GLMM with weight factor, interaction: $p<0.01$. See Table 7.2). Residuals were normally distributed.

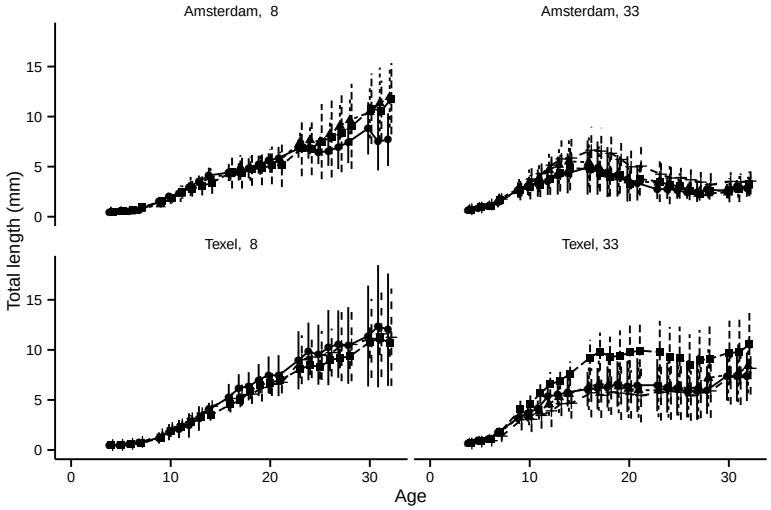
The onset of metamorphosis and the size of the oral lobes differed between groups but showed high variation (Fig. 7.6). The high salinity groups both started developing lobes at an age of 11 days, but lobe formation in both low salinity groups started 4 days. Directly after the start of lobe forming, the TL/OA ratio was similar for groups on the same salinity, while differences started to occur after 5-8 days. After 15 days the TL/OA ratio for Amsterdam high salinity decreased, which corresponded with the shrinking described previously. This decrease coincided with the observation that individuals of the Amsterdam group on high salinity were mostly found on or near the bottom and had damaged lobes.

Reproduction

The first eggs were observed on day 21 in the Amsterdam low salinity group. This group also produced the most eggs per individual during the experiment (Fig.



(a) mean and sd of OA length per replicate



(b) mean and sd of total length per replicate

Figure 7.5: Mean and standard error of oral-aboral (OA, upper four panels) and total (lower four panels) length (mm) for each container in the experiment. Different symbols represent the different replicates. Points are slightly offset to show the overlapping standard errors.

Table 7.1: Mean (with standard error), minimum and maximum oral-aboral and total lengths (mm) after 32 days. AM: Amsterdam, TX: Texel. 8 and 33 are the salinity levels.

	Oral-aboral length			Total length		
	Average	Min	Max	Average	Min	Max
AM33	2.6 ± 0.7	1.8	4.6	3.2 ± 1.1	1.8	6.0
AM8	7.5 ± 2.0	3.8	11.5	10.7 ± 3.5	4.2	16.0
TX33	5.8 ± 1.5	3.2	8.6	8.6 ± 3.0	4.4	14.3
TX8	7.5 ± 2.3	3.2	12.0	11.3 ± 4.2	4.2	19.0

Table 7.2: Model results of the weighted GLMM of oral-aboral lengths at the end of the experiment versus salinity and location. AM: Amsterdam, TX: Texel. 8 and 33 are the salinity levels.

	AM33	AM8	TX33	TX8	
Parameter estimates	1.00	3.06	2.21	3.51	
	Value	SE	df	t-value	p-value
Intercept	7.52	0.54	85	13.83	<0.01
Location Texel	-0.04	0.78	10	-0.04	0.96
Salinity 33	-4.92	0.56	10	-8.74	<0.01
Interaction	3.27	0.83	10	3.93	<0.01

7.7). The maximum observed daily egg production per individual was 1 for the Amsterdam 33 group, 24 for the Amsterdam low salinity group, 3 for the Texel high salinity group and 5 for the Texel low salinity group. The average reproduction per individual per day differed between groups (negative binomial GLM; interaction: $p < 0.01$, see Table 7.3). After validation, this model still showed overdispersion (dispersion coefficient = 2.18). A sampling error (taking a too small subsample) prevented accurate measurement of egg production on day 24, so values from this day are excluded.

Carbon content

Individual carbon content at the end of the experiment differed between groups as well (Table 7.4). Carbon fraction for both low salinity groups was much higher compared to high salinity groups, but there were no differences between locations (Table 7.5).

Genetic structure

After correction for Benjamini-Hochberg False Discovery Rate (B-H FDR), HWE (adjusted $\alpha = 0.002$) and LD (adjusted $\alpha = 0.002$) showed 2 and 3 significant

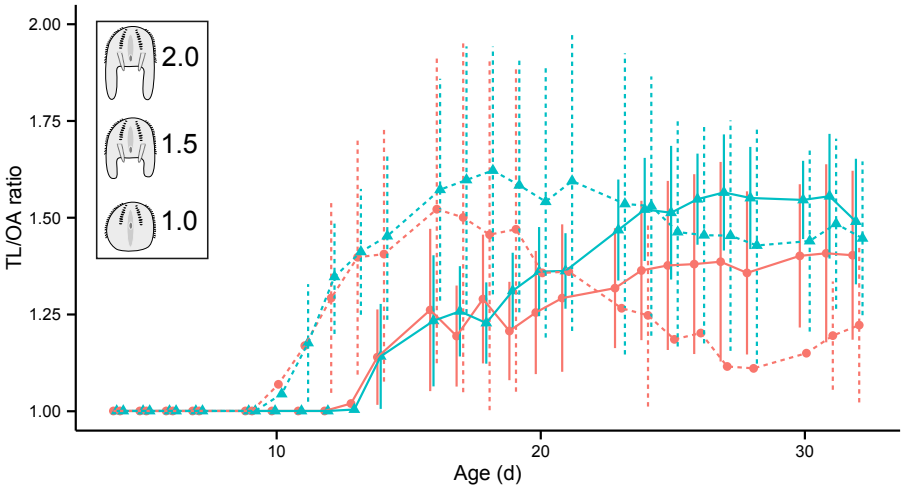


Figure 7.6: Mean and standard error of the ratio between total length and oral-aboral length for each group. Red lines and circles: Ctenophores collected in Amsterdam, Blue lines and and triangles: ctenophores collected on Texel. Solid line: low salinity (8), dashed line: high salinity (33). Points are slightly offset to show overlapping standard errors. As an example, the general shape of animals with a TL/OA ratio of 1.0, 1.5 and 2.0 is given.

deviations for HWE (7.1 %) and LD (3.6 %), respectively. Deviations were neither uniform across sub-populations (LD), treatments nor loci.

Pairwise F_{ST} values indicate genetic structure after high and low salinity treatments of the two *Mnemiopsis leidyi* sub-populations. The largest genetic differentiation, hence highest F_{ST} values (0.13-0.23, p < 0.0001) were observed between salinity treatments among sub-populations. On the other hand, genetic differentiation between salinity treatments within sub-populations was much lower (table 7.6). Significance level before and after B-H FDR correction was 0.05 following (Benjamini and Yekutieli, 2001; Narum, 2006). Overall, high and low salinity treatments of the Texel sub-populations revealed the lowest genetic variation (0.064, p = 0.0001). Similarly, AMOVA results show that the majority of genetic variation occurs within sub-populations (>80 %, p < 0.0001, Table 7.7). However, 10.4 % (p=0.0006) of the variation is explained by the among group difference, hence origin of sub-populations from either Texel or Amsterdam with salinity effects being clustered underneath explaining 7.6% (p < 0.0001) of the variation. On the other hand, when conducting a hierarchical grouping based on salinity, this term becomes non-significant (p = 0.9) and the among sub-populations within groups variation, hence origin of sub-population explains 19.3 % (p < 0.0001) of the variation (for F-statistics, see table 7.7).

STRUCTURE analyses clustered the samples in 3 groups (k = 3) (Evanno *et al.*, 2005). Two of these clusters are associated with salinity treatments of the Amsterdam sub-population, while the other cluster includes all samples of the Texel

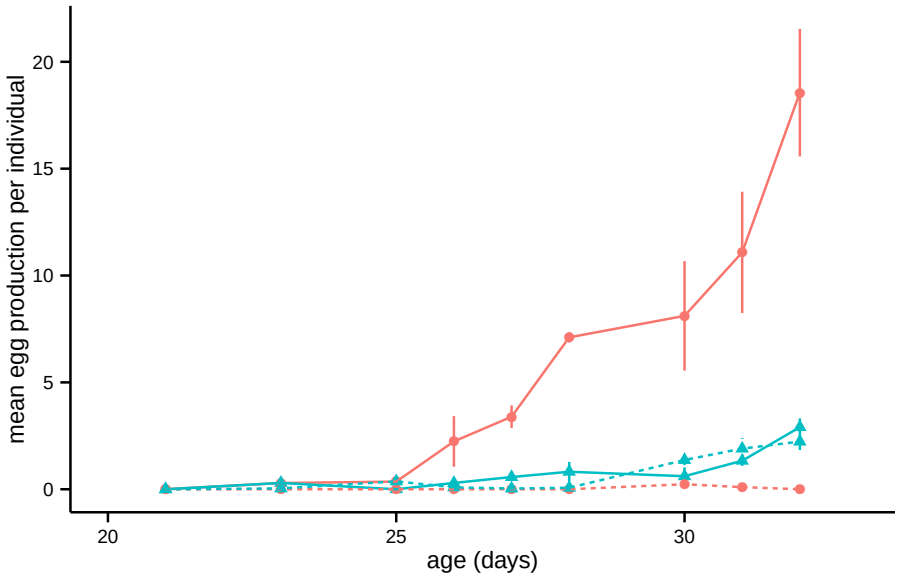


Figure 7.7: Mean and standard error of egg production for each group. Red lines and circles: Ctenophores from Amsterdam, Blue lines and and triangles: ctenophores from Texel. Solid line: low salinity (8), dashed line: high salinity (33). A sampling error (taking a too small subsample) prevented accurate measurement of egg production on day 24, so values from this day are excluded.

sub-population (Fig. 7.8).

When investigating number of alleles between both sub-populations, the Texel sub-population has a lower mean number of alleles (5.6±3.1; ± sd), than the Amsterdam sub-population (7.1±3.2), with the highest mean number of alleles recorded in the low salinity treatment group of the Amsterdam sub-population.

Table 7.3: Model results for the binomial GLM of egg production versus location and salinity.

	Estimate	SE	z-value	$Pr(>\|z\|)$
Intercept	1.84	0.17	10.65	<0.01
Location Texel	-1.67	0.25	-6.59	<0.01
Salinity 33	-4.70	0.34	-14.00	<0.01
Interaction	4.23	0.41	10.20	<0.01
	df	value		
Null deviance	13	291.3		
Residual deviance	10	21.7		
AIC		129.9		

Table 7.4: mean ±SE of dry weight, carbon and nitrogen-content and -fraction.

S	Area	n	dry weight (µg)	C (µg)	fraction C	N (µg)	fraction N
8	Amsterdam	3	9.077 ±2.073	0.769 ±0.148	0.087 ±0.012	0.185 ±0.036	0.021 ±0.003
8	Texel	3	10.793 ±2.415	0.811 ±0.139	0.077 ±0.006	0.187 ±0.032	0.018 ±0.001
33	Amsterdam	6	1.672 ±0.347	0.049 ±0.011	0.029 ±0.001	0.010 ±0.002	0.006 ±0.000
33	Texel	4	22.152 ±2.083	0.499 ±0.057	0.022 ±0.001	0.107 ±0.013	0.005 ±0.000

Table 7.5: Analsis of variance table of carbon fraction per individual at the end of the experiment.

	Df	Sum Sq.	Mean Sq.	F value	$Pr(>F)$
Area	1	21.16	21.16	0.23	0.6382
Salinity	1	12070.79	12070.79	132.70	0.0000
Area:Salinity	1	12.25	12.25	0.13	0.7200
Residuals	12	1091.52	90.96		

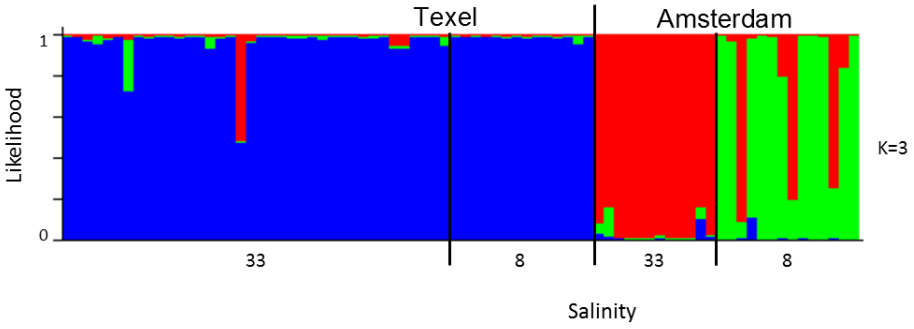


Figure 7.8: Bar plots of *Mnemiopsis leidyi* genotypes from two different sub-populations (Texel and Amsterdam), after culturing at high (33) and low (8) salinity treatments for 32 days. Results of Bayesian clustering analyses using STRUCTURE with k=3.

Table 7.6: Pairwise genetic differentiation matrix (F_{st}) for cohort experiments of two *Mnemiopsis leidyi* sub-populations (Texel, TX and Amsterdam, AM), cultured for 32 days at high (33) and low (8) salinity levels, respectively. Significant values highlighted in bold (p< 0.001**, p < 0.0001***, adjusted α = 0.05

	TX33	TX8	AM8
TX8	0.064**	-	
AM8	0.151***	0.226***	-
AM33	0.130***	0.205***	0.077***

Table 7.7: Analysis of molecular variance (AMOVA) analyses for *Mnemiopsis leidyi* from 2 source populations (Texel and Amsterdam) held at two salinities (33 and 8). Results show partitioning of genetic variation using hierarchical structure with 1) sampling location (groups) as highest order and salinity treatment (populations) nested within sampling location and 2) salinity (groups) as highest order and sampling location (populations) nested within salinity treatment.

Group	Source of variation	Sum sq.	Var. comp.	% of var.	p-value	F-Statistic
1	Among groups	28.01	0.28	10.38	0.0006	F_{CT} -0.06
	Among populations within groups	17.42	0.21	7.57	<0.0001	F_{SC} 0.182
	Within populations	320.01	2.20	82.05	<0.0001	F_{ST} 0.133
2	Among groups	10.54	-0.15	-6.04	0.9	F_{CT} 0.104
	Among populations within groups	34.88	0.49	19.32	<0.0001	F_{SC} 0.085
	Within populations	320.01	2.20	86.72	<0.0001	F_{ST} 0.180

Discussion

Our common garden experiments shows that *Mnemiopsis leidyi* from two different sub-populations in Northern Europe can be reared at low salinity in the laboratory. However, mortality rates in the low salinity (8) treatment were very high and more than two thirds of the animals died during the first 6 days after hatch. Previous studies investigated the effect of low salinity on feeding and egg production, testing salinities between 5.7 and 33 (Jaspers *et al.*, 2011; Lehtiniemi *et al.*, 2012; Hosia *et al.*, 2012). These studies used either cultured animals which were gradually acclimatised to the target salinity over ca. 1 to 2 weeks and kept at those salinities for > 1 week before start of the experiments, using salinity acclimatised food (Jaspers *et al.*, 2011; Hosia *et al.*, 2012) or by using field collected animals employing a <1 day salinity change and acclimatization period before start of the experiments (Lehtiniemi *et al.*, 2012). These studies showed that salinities <10 had a dramatic effect on physiological rates suggesting that the low salinity range of the Baltic Sea sets a limit to their range expansion (Jaspers *et al.*, 2011; Lehtiniemi *et al.*, 2012).

The growth patterns observed for Amsterdam on low salinity and the Texel groups were similar to a recent growth study (Jaspers, 2012), but lengths observed were smaller at similar ages than that of older studies which were conducted at higher temperatures ranging from 21 to 26 °C (Baker and Reeve, 1974; Kremer and Reeve, 1989; Reeve *et al.*, 1989).

Invasion models often use data from several different sources to estimate parameters for growth survival, reproduction in relation to environmental variables to predict invasion risks for new areas, such as Salihoglu *et al.* (2011),Collingridge *et al.* (2014) and Augustine *et al.* (2014a) for *M. leidyi*. However, in our study we show that a low saline tolerant*M. leidyi* sub-population is present in northern Europe. This highlights that it is important to realize that (sub)populations of invaders from different areas do not alway show the same phenotypical response to differing environmental conditions. It is thus important to validate model assumptions with physiological rate measurements from the different sub-populations in question since they may show a different environmental tolerance window.

Differences between salinities

Survival differed between different salinity treatments, especially in the larval stage. The high salinity treatment of the low saline Amsterdam population origin lead to highest mortality rates observed throughout the experiment. Martindale (1987) found highly varying survival rates ranging from 6–89 % after ten days at an unknown salinity level. (Lehtiniemi *et al.*, 2012) found no survival at salinities lower than 10, while half of the animals survived at a salinity of 30. As the present experiment was started with 4-day old larvae, mortality occuring in the first four days preceding the experiment was unknown, but was likely high. Despite this, the experiment showed that larval survival is possible at a salinity as low as 8.

The mechanism by which salinity influences *M. leidyi* phenotypical traits is unknown. Gelatinous zooplankton apear to be mainly osmoconformers (Mills, 1984) and need a certain period of time to regain their equilibrium buoyancies

when transferred to water with a different salinity. This does not mean however that the ion composition inside the animals is the same as that in the surrounding seawater. Most gelatinous zooplankton species use exclusion of heavy sulphate ions as the main source of buoyancy (Mills, 1984). *M. leidyi* appears to be an osmoconformer as well, but here as well the ion composition of the body differs from that of the water column (Foshtomi *et al.*, 2007).

One explanation could be that the phenotypical response to different salinity levels differs for different temperature levels. This interaction has not been investigated so far in *M. leidyi* or other gelatinous zooplankton species, but has been studied in other marine invertebrates. In several crustacean taxa it has been shown that osmoregulatory capacity differs between temperature levels (Mantel and Farmer, 1983; Lemaire *et al.*, 2002), and toxicity of heavy metals increases with lower salinity levels (Jones, 1975; McLusky and Hagerman, 1987; Bambang *et al.*, 1995) because these toxicants impair the osmoregulatory capacity of the animals.

Egg production in this study was already observed at an age of 21 days at an average ctenophore size of 5 mm in the Amsterdam low salinity group (Fig. 7.5(a)). At this age, most of the ctenophores had already started forming lobes (Fig. 7.6). In their native range, the average size at first reproduction is 30 $\pm$ 5 mm at an age of 13 to 17 days (Baker and Reeve, 1974), but reproduction by cydippid larvae sized 1.5–2.8 mm has been observed for *M. leidyi* (Martindale, 1987) and other ctenophore larvae (Chun, 1892; Jaspers *et al.*, 2012). Larval growth and reproduction appears to be very variable in *M. leidyi*, (as reviewed in Augustine *et al.*, 2014a) and delaying reproduction under low food conditions has been hypothesised to be a strategy to survive periods of low food availability (van der Molen *et al.*, 2015).

Differences between origin of sub-populations

Growth patterns of the Amsterdam high salinity group were also strikingly different. Initially individuals had the same growth pattern as individuals from the Texel, but after 15 days they started to shrink. The shrinking started with regression of the lobes. There could be several explanations for this. Starvation is one, as the animals in the Amsterdam high salinity group shrank and had a very low carbon content at the end of the experiment. Food concentrations and volume adjustments were the same for all treatments, but the regression of the lobes in the Amsterdam high salinity group could have impaired their feeding capacity.

This can be caused by several factors: (1) the group suffered from an infection, (2) food concentrations were too low, (3) the animals were damaged during handling or (4) that a positive selection on standing genetic variation for low saline tolerance in the Amsterdam population lead to low tolerance against high salinity. An infection could be the cause, but is not likely because the shrinking only appeared in one group and not in any of the others. Containers were randomly assigned and were placed in a mixed configuration. The Amsterdam population at high salinity showed an opposite pattern in reproduction and growth characteristics compared to so far published results from northern Europe (Jaspers *et al.*,

2011). This might indicate that this genotype caught in low salinity is either adapted to low salinity and looses the ability to cope with higher salinities or that this sub-population is characterised by a large standing genetic variation allowing for high growth and egg production under low salinity levels due to genotypic sorting.

For the low salinity groups the growth patterns were similar, but the Amsterdam group kept on the low salinity started reproduction earlier and produced much more eggs during the experiment than the Texel group, showing that there was indeed a difference between sub-populations; animals originating from a low salinity area produced more eggs at their "native" salinity level.

Genetic basis for low salinity tolerance?

It would have been interesting had the experiment been continued for several more weeks, but due to time constraints and a limit to the amount of *A. tonsa* copepods available as food this was not possible. The egg production of all groups would have likely increased several orders of magnitude as reproduction is a function of size (Kremer, 1976a; Jaspers *et al.*, 2011). It can not be excluded that egg production at higher salinities starts at older ages or larger sizes than at lower salinities.

The individuals used for the Amsterdam group were caught in the North Sea Canal area close to the city of Amsterdam. Although the salinity at the surface was measured when the animals were collected was 6–8, the North Sea Canal is stratified (van Banning *et al.*, 2011). The salinity gets higher in the deeper parts and closer to the North Sea. Higher survival and reproduction in the deeper layers could contribute to the blooms observed. An investigation of the vertical distribution of *M. leidyi* in the canal, as for instance done by Haraldsson *et al.* (2013) in the Baltic, is necessary to confirm this Jaspers *et al.* (2011).

These results show that *M. leidyi* can survive, grow and reproduce at lower salinity levels than suggested before. This could mean that the species could spread to low-saline regions currencty deemed to be unsuitable habitat for *M. leidyi* populations, like the northern and central Baltic Sea (Jaspers *et al.*, 2011; Haraldsson *et al.*, 2013).

The difference shown in reproduction between the two locations studied, especially between the low salinity groups, can not be explained by phenotypic plasticity.

Our genetic results show that *M. leidyi* individuals, which were collected from two invasive sub-populations and exposed to 2 different salinity treatments, show significant differences in their genetic structure. First, allele frequencies of the Texel and Amsterdam *M. leidyi* sub-populations are highly differentiated (F_{st} ranging between 0.13–0.22). Comparing these results with a recently published world-wide phylogeography of *M. leidyi* indicates that our genetic differentiation is much larger than previously observed in natural populations of northern Europe (Bayha *et al.*, 2015). Bayha *et al.* (2015) conclude that North Sea and Baltic Sea *M. leidyi* sub-populations show no differentiation between both regions (pairwise F_{st} values -0.007, non-significant).

Similarly, another study showed no differentiation between North Sea and Skagerrak sub-populations (pairwise F_{st} = 0.003, non-significant) (Reusch *et al.*, 2010). Therefore, high F_{st} values found in this study between Amsterdam and

Texel sub-populations indicate strong genetic structure and separation between both sub-populations being nearly 2 orders of magnitude larger compared to so far published investigations in Northern Europe. The differentiation found here (this study) is similar to genetic structure found between Gulf of Mexico and NE USA *M. leidyi* sub-populations (Reusch *et al.*, 2010; Bayha *et al.*, 2015).

This strong genetic structure indicates reproductive isolation between Amsterdam and Texel sub-populations, even though they are geographically very close (less than 70 km). This could be explained by the long residence time of water within the Amsterdam Port. Interestingly, we observed a sub-structure within the Amsterdam sub-population where animals from the two salinity treatments showed a significant difference in allele frequency, which also showed up as an additional cluster in the structure analyses. This low saline Amsterdam sub-population showed highest overall higher size specific reproduction rates. Since higher allelic richness was found in the Amsterdam sub-population, the salinity treatment could have led to a sorting of genotypes. The area receives 7,000–9,000 seagoing ships annually (Port of Amsterdam, 2013) and it might be that re-current invasions due to frequent ballast water discharge could be an important component explaining this pattern.

The here presented results confirm the presence of *M. leidyi* individuals with a low salinity tolerance in Northern Europe. This result urges for future investigations and combined efforts for ballast Water treatment to prevent spread of this genotype to low saline regions.

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Chapter 8

Trophic overlap of the invasive ctenophore *Mnemiopsis leidyi* with other zooplanktivores in the western Dutch Wadden Sea

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Abstract

In this study the isotopic position and niche of the invasive ctenophore *Mnemiopsis leidyi* was determined by analysing the diet of macroplankton and nekton species of the western Wadden Sea from monthly samples in March–August, 2011 in the Marsdiep area and adjacent tidal gullies. Stable carbon and nitrogen isotope signatures of fish, scyphozoa, hydromedusa, ctenophores, crustaceans and cephalopods were determined. A cluster analysis showed that average δ^{13}C and δ^{15}N ratios of the invasive *Mnemiopsis leidyi* were similar to those of most other gelatinous zooplankton species and fish species of intermediate trophic level.

Estimation and comparison of isotopic niches for each species in each month showed that the isotopic niche of *M. leidyi* overlaps with that of fish species such as the glass goby *Aphia minuta*, the herring *Clupea harengus* and the horse mackerel *Trachurus trachurus* as well as with that of gelatinous zooplankton species such as the compass jellyfish *Chrysaora hysoscella*, the sea gooseberry *Pleurobrachia pileus* and the hydroid *Nemopsis bachei* in the spring and early summer period, when *M. leidyi* densities are low.

δ^{15}N of *M. leidyi* was positively related to ctenophore size, suggesting that small ctenophores occupy a lower trophic level than large ones. At the beginning of the bloom period in August when almost the entire population consisted of larvae and juveniles there was no overlap in isotopic niche of *M. leidyi* with that of any other pelagic zooplanktivore. The period of high diet overlap with other consumers is also the period in which *M. leidyi* is least abundant. This suggests that at present, *M. leidyi* is not a significant competitor for other gelatinous zooplankton and fish species. During the bloom period of *M. leidyi* the abundance of competing species is low, suggesting that *M. leidyi* is using an unoccupied niche.

Introduction

Mnemiopsis leidyi is an opportunistic planktonic predator of western Atlantic coastal waters which feeds on a wide range of different zooplankton prey such as copepods, copepodites and nauplii, bivalve veligers, barnacle nauplii and cyprids (Granhag *et al.*, 2011; Javidpour *et al.*, 2009b), fish larvae (Cowan and Houde, 1992) and eggs (Purcell *et al.*, 1994).

For several decades now, *Mnemiopsis leidyi* has been observed outside of its native range as invasive species. The first invasion of *M. leidyi* was in the Black Sea, where it was first found in 1982. After 1989 density and biomass of the species reached very high levels following recruitment failure in the dominant zooplanktivorous fish species, the anchovy *Engraulis encrasicolus* (Bilio and Niermann, 2004) due to overfishing. A lack of competition, eutrophication and climate induced enhanced carrying capacity led to a competitive advantage of *M. leidyi* over pelagic fish (Oguz *et al.*, 2008). Fisheries in the Black Sea region suffered heavy economic losses because of the collapse of pelagic stocks (Knowler, 2005).

Recently the invasive ctenophore *Mnemiopsis leidyi* has also been reported from many different areas in western Europe: Sweden (Hansson and Kiørboe, 2006), Germany (Javidpour *et al.*, 2006; Boersma *et al.*, 2007), Denmark (Tendal *et al.*, 2007), the Baltic Sea (Javidpour *et al.*, 2006), Poland (Janas and Zgrundo, 2007) Norway (Oliveira, 2007) and the Dutch coast (Faasse and Bayha, 2006; Tulp, 2006) including the Wadden Sea where it occurs year-round in the western Wadden Sea in significant numbers, especially in summer and autumn (Van Walraven *et al.*, 2013).

Previous studies estimated the grazing pressure of gelatinous zooplankton on mesozooplankton prey in the Wadden Sea to be low (Van der Veer and Sadée, 1984; Van der Veer, 1985; Daan, 1986). The introduction of *M. leidyi* warrants a closer evaluation of the role of gelatinous zooplankton as predators of mesozooplankton in the area. In the German part of the Wadden Sea *M. leidyi* had a high overlap in diet with the zooplanktivorous fish *Clupea harengus* (Kellnreitner *et al.*, 2013). Based on gut content analysis of Wadden Sea fish species, several other species are known to have similar diets to *C. harengus* (Kellnreitner *et al.*, 2012).

Comparing diets of different animals using gut content analysis can be difficult. Especially jellyfish and other gelatinous zooplankton often have fast digestion rates (Pitt *et al.*, 2009; Purcell, 2009), and digestion time varies for different prey species (Purcell, 1991a). In *M. leidyi* digestion times ranged from 0.4 hours for Tintinnid ciliates to 4.8 hours for *Acartia tonsa* copepods (Granhag *et al.*, 2011).

One method that has been used as an addition to gut content analysis is Stable Isotope Analysis (SIA). Stable Isotope Analysis has been used for the analysis of diet of gelatinous zooplankton in in a wide range of ecosystems (Malej *et al.*, 1993; Brodeur *et al.*, 2002; Pitt *et al.*, 2008; Frost *et al.*, 2012) as well as freshwater and marine fish (Gu *et al.*, 1996; Jennings *et al.*, 2002; Post, 2002; Brodeur *et al.*, 2002). Not often do studies include both gelatinous zooplankton and fish in their sampling but several studies that did compare jellyfish stable isotope ratios with those of pelagic fish show that the diet of the two groups can overlap (Brodeur *et al.*, 2002; Kellnreitner *et al.*, 2013) or reveal fishes as predator of jellyfish (Utne-Palm

et al., 2010).

The goal of this study is to gain insight in the trophic position of the invasive ctenophore *Mnemiopsis leidyi* compared with native fish and gelatinous zooplankton species. The following research questions are discussed:

- What is the trophic position of gelatinous zooplankton and pelagic fish in the Dutch Wadden Sea and does this change during the year?
- Which gelatinous zooplankton species have an overlapping diet with the invasive ctenophore *Mnemiopsis leidyi*?

Material and methods

Field sampling

The sampling survey took place monthly from March–August 2011 in the westernmost tidal basin of the Wadden Sea, the Marsdiep. Several types of sampling gears were used to collect gelatinous zooplankton as well as fish. Along a transect in the Marsdiep area oblique hauls of 10 minutes were made using an Isaacs-Kidd midwater trawl with a trawling speed of 2 knots. The net had a mouth opening of 4 m^2 and a mesh size of 5 mm in the back part.

At fixed stations in tidal gullies of the Balgzand intertidal additional samples were taken every one or two weeks using plankton nets made of polyamide plankton gauze (Monodur 2000, 2 mm mesh size) with an opening of 0.7 m^2, a length of 5 m, a porosity of 0.59 and a total surface area of 12 m^2. Oblique hauls were made with the ship at anchor in the tidal current. For a detailed description of the area and methods see the previous chapter .These stations were similar to the ones sampled in 1981-1982 (Van der Veer and Sadée, 1984; Van der Veer, 1985) and 2009 (Van Walraven *et al.*, 2013).

On board catches were sorted and all animals present, or in a subsample, were measured to the nearest mm according to Table 8.1.

Table 8.1: Measurements used for different groups. All measurements were in mm.

group	measurement
Fish	total length
Scyphozoa	bell diameter
Athecate hydromedusae	bell height
Thecate hydromedusae	bell diameter
Beroid and cydippid ctenophores	polar length
Lobate ctenophores	oral–aboral length
Other invertebrates	total length

Stable isotope analysis

On each survey at least 5 individuals of each species were collected if possible for stable isotope analyses of δ^{15}N and δ^{13}C.

Bell tissue was used for scyphomedusae as δ^{13}C and δ^{15}N of the bell appears to be representative for that of the whole body in *Aurelia aurita* (d'Ambra *et al.*, 2014). For ctenophores, except for smaller sized *M. leidyi* (<15 mm), tissue around the stomach and statocyst was removed to prevent contamination. *M. leidyi* smaller than 15 mm were placed into filtered seawater for 2–4 hours for digestion of possible food and the whole individual was used. Back muscle tissue was used for fish (Pinnegar and Polunin, 1999). For small fish, larvae and some species such as the pipefish *Syngnathus rostellatus* excision of the back muscle tissue was not possible and a section of tail without gonads, stomach and intestines was used.

Two plankton fractions were collected as well: seawater was collected using a bucket and filtered over a 80 µm sieve after which the filtrate was filtered through a GF/F filter.

All samples were stored in glass vials at -20 °C until freeze drying. Samples were freeze dried for at least 24 hours until constant weight.

At least 1 mg (fish and non-gelatinous plankton) or 10 mg (gelatinous zooplankton) was put in 9x5 mm tin cups. Freeze-dried and encapsulated samples were analysed for δ^{13}C and δ^{15}N SI ratios using a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer equipped with a Flash 2000 Organic Element Analyser at the Royal Netherlands Institute for Sea Research, Texel, Netherlands.

l-Glutamic acid (δ^{15}N -4.36 ‰, δ^{13}C -26.2 5‰, 40.81 % C, 9.52 % N) and Acetanilide (δ^{15}N 1,3 ‰ δ^{13}C -26,1 ‰, 71.08 % C, 10.36 % N) were used as reference material for quantification of C and N ratios, respectively (Schimmelmann *et al.*, 2009; Qi *et al.*, 2003). The ^{13}C composition was expressed relative to the level in Peedee-Belemnite limestone:

$$\delta^{13}\mathrm{C} = \left(\frac{\mathrm{R}_{sample}}{\mathrm{R}_{standard}} - 1 \right) * 1,000 \tag{8.1}$$

with R being the ratio ^{13}C:^{12}C. The ^{15}N composition was expressed relative to the level in atmospheric N_2 using the same formula with R being the ratio ^{15}N:^{14}N.

Samples were analysed in duplicate unless insufficient material was available. Samples from individuals with a standard deviation larger than 1 ‰ for δ^{13}C and larger than 2.5 ‰ for δ^{15}N were excluded from the analysis.

As lipid content can influence δ^{13}C values in aquatic animals, especially at higher lipid contents (Post *et al.*, 2007), δ^{13}C values were corrected for lipid content. The carbon-to nitrogen ratio (C:N) by mass of the sample as determined during analysis for δ^{13}C and δ^{15}N was used to apply the correction described in Post *et al.* (2007):

$$\delta^{13}\mathrm{C}_{normalised} = \delta^{13}\mathrm{C}_{untreated} - 3.32 + 0.99 * \mathrm{C{:}N} \tag{8.2}$$

Data analysis

Relative Trophic Position (RTP) was estimated using the equation:

$$\text{RTP} = \left(\delta^{15}\text{N}_{\text{Consumer}} - \delta^{15}\text{N}_{\text{base}}\right)/3.4 + 2 \tag{8.3}$$

assuming that the RTP of the long-lived primary consumer used as the baseline was 2 and δ^{15}N was enriched by 3.4 ‰ for each higher trophic level (Minagawa and Wada, 1984; Post, 2002). The primary consumer used as baseline was a filter-feeding bivalve, as used in similar studies (Post, 2002; Kellnreitner *et al.*, 2012), blue mussels *Mytilus edulis*, were collected from the tidal flats of the Mokbaai intertidal area.

Stable isotope ratios of consumers were compared in two ways. Average values were compared by performing a cluster analysis, and within and between-species variation was investigated using the Standard Ellipse Area method as described below.

A cluster analysis was performed on all consumer isotope data collected in 2011 to investigate general patterns and similarity in δ^{15}N and δ^{13}C values between species, which has been used in various marine systems before (Davenport and Bax, 2002; Grall *et al.*, 2006; Nagata *et al.*, 2015). This analysis was similar to the one used in Nagata *et al.* (2015). A matrix of Euclidean Metric Distances (EMD) was created from mean δ^{15}N and δ^{13}C values of each species with the main clusters identified at an EMD of < 2.5.

Variation in trophic position and niche width of consumers was investigated by calculating the Standard Ellipse Area corrected for small sample sizes (SEA_c) from δ^{15}N and δ^{13}C measurements of individuals, both over the whole sampled period and per month. This is a bi-variate measure of variation similar to the standard deviation (Jackson *et al.*, 2011).

Possible diet overlap between *Mnemiopsis leidyi* and other consumers was investigated by calculating the percentage of overlap of the area of the SEA_c ellipse of the various consumers with that of *M. leidyi*. The estimation of SEA_c and overlaps were performed using the SIBER routines (Jackson *et al.*, 2011) found in the R package SIAR, version 4.2 (Parnell *et al.*, 2010).

For *Mnemiopsis leidyi* the relationship between ctenophore oral-aboral length in mm and δ^{15}N for different months was investigated using a linear regression model of the form:

$$M1 : \delta^{15}\text{N}_{ij} = length_i + month_j + \epsilon_{ij} \tag{8.4}$$

whereby stepwise backwards selection was performed to find the optimal model.

All analyses were performed in R 3.1.3 (R Core Team, 2014).

Results

δ^{15}N and δ^{13}C ratios were obtained for 683 individuals of 36 different species on 26 different sampling days. Most species caught were fish (19 species). 13 species of gelatinous zooplankton were caught: four ctenophores, five scyphomedusae and

four hydromedusae. Two amphipods and two mysid species were also sampled (Table 8.3). Most bulk plankton samples did not analyse properly on the IRMS because the amount of C and N in the samples was too low, and data on the two plankton fractions could only be obtained for August. The baseline δ^{15}N values from long-lived filter-feeding primary consumers, the bivalve *Mytilus edulis* were collected in March and July 2014 in the Mokbaai (53.0041 N, 4.7711 E) and had a mean δ^{15}N value of 10.8 ‰ and a SE of 0.1 ‰ (n=15). Mean δ^{15}N values of *Mytilus edulis* did not differ between March and July (one-way ANOVA, $F_{(1,13)}$=1.503, p=0.242) and were similar to mean δ^{15}N values found for the $>$ 80 μm plankton fraction (one-way ANOVA, $F_{(1,17)}$=1.133, p=0.302).

Relative Trophic Positions

Average Relative Trophic Positions (RTPs) of all species are shown in Table 8.3 and Fig. 8.1.

The squid *Loligo vulgaris* had the highest average RTP at 4.1, followed by several fish species such as the bib *Trisopterus luscus*, the hooknose *Agonus cataphractus*, the whiting *Merlangius merlangius* and the smelt *Osmerus eperlanus*. The gelatinous animal with the highest RTP was *Aequorea vitrina*, which had the 12^{th} highest RTP overall; 3.4. The scyphomedusae species with the highest RTP was *Chrysaora hysoscella* at 3.0. The other scyphomedusae species had a surprisingly low RTP of less than 3.

Average RTP of *Mnemiopsis leidyi* was 3.1, close to that of *Pleurobrachia pileus* (3.2), *Beroe gracilis* (3.1) and *Beroe cucumis* (3.0). Gelatinous zooplankton species with the lowest RTP were the hydromedusae *Sarsia tubulosa* and *Cosmetira pilosella*. The lowest RTP values were found for the $<$ 80 µm plankton fraction (1.3) which was close to the minimum value of 1 for autotrophs. The mixotrophic dinoflagellate *Noctiluca scintillans* had a RTP of 1.9.

Isotopic niches of taxa

Species were grouped according to taxonomical group. For these groups the isotopic niche was estimated by calculating the Standard Ellipse Area corrected for small sample size (SEAc, Fig. 8.2 and Table 8.2). The isotopic niche of fish overlapped most with that of the cephalopods and ctenophores, and least with that of scyphozoa and crustacea. Fish had the largest isotopic niche, followed by the ctenophores, hydromedusae, scyphomedusae, crustacea and cephalopods.

Clusters

Seven main clusters were identified (Fig. 8.3). All ctenophores and scyphomedusa clustered together with most pelagic fish species of intermediate trophic position (Fig. 8.1) including herring *Clupea harengus* and sprat *Sprattus sprattus* in cluster A. This cluster also included the squid *Alloteuthis subulata*, the parasitic amphipod *Hyperia galba*, an isopod (*Idotea linearis*) and a mysid (*Gastrosaccus spinifer*).

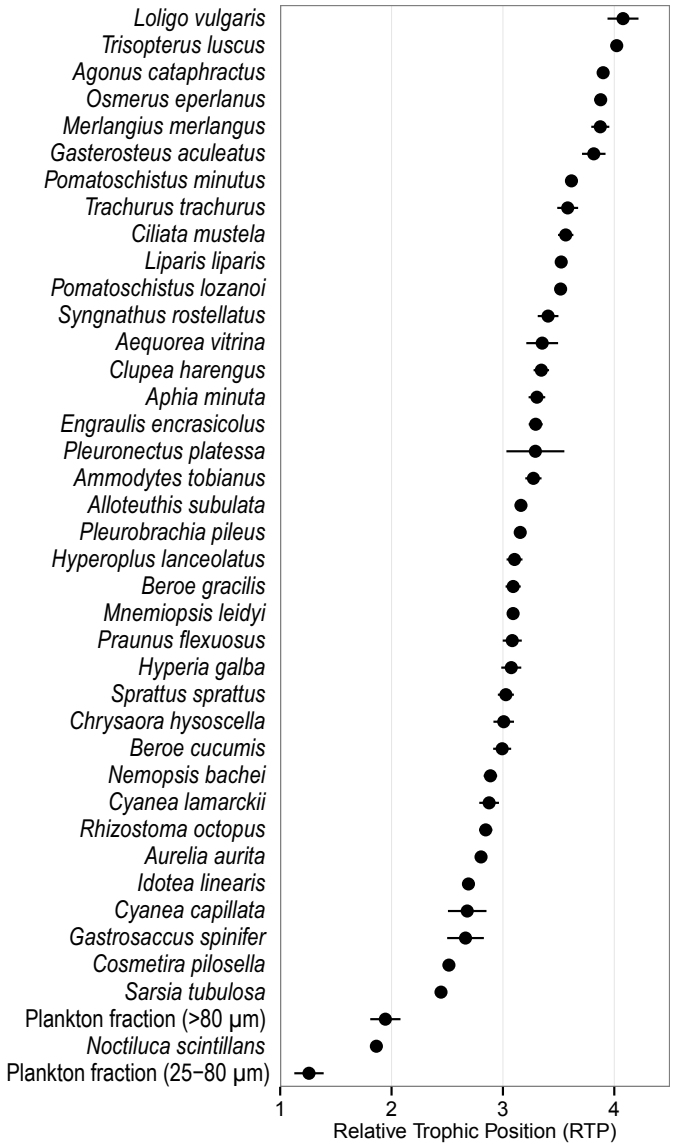

Figure 8.1: Mean Relative Trophic Position (RTP) per species in 2011, ordered highest to lowest.

Table 8.2: Standard Ellipse Area corrected for small sample sizes (SEA_c, as δ units^2) and percent overlap of the SEA_c of groups of consumers with each other.

	SEA_c	Overlap (%)					
		Ceph	Crus	Cten	Fish	Hydr	Scyp
Cephalopods (Ceph)	0.43		81	95	98	58	58
Crustacea (Crus)	4.10	9		43	16	32	48
Ctenophora (Cten)	6.22	7	29		55	59	53
Fish	9.21	5	7	37		13	9
Hydromedusae (Hydr)	6.13	4	22	60	19		71
Scyphozoa (Scyp)	5.22	5	37	63	16	84	

Table 8.3: Sizes and means and standard error of δ^{13}C, δ^{15}N, Relative Trophic Position (RTP) and C:N ratio,per species over the whole sampled period in 2011.

Species	n	size mm		δ^{13}C ‰		δ^{15}N ‰		RTP		C:N	
		min	max	mean	SE	mean	SE	mean	SE	mean	SE
Aequorea vitrina	6	35	81	-16.6	0.8	15.4	0.5	3.4	0.1	3.5	0.1
Agonus cataphractus	1	45	45	-17.5		17.2		3.9		3.3	
Alloteuthis subulata	5	47	88	-18.0	0.3	14.7	0.2	3.2	0.1	3.7	0.0
Ammodytes tobianus	18	44	182	-18.3	0.2	15.1	0.2	3.3	0.1	3.4	0.1
Aphia minuta	17	25	57	-18.3	0.2	15.2	0.3	3.3	0.1	3.5	0.0
Aurelia aurita	38	23	251	-18.5	0.2	13.5	0.2	2.8	0.0	3.4	0.1
Beroe cucumis	26	15	70	-18.5	0.3	14.1	0.3	3.0	0.1	3.9	0.0
Beroe gracilis	25	12	34	-19.4	0.5	14.5	0.2	3.1	0.1	3.8	0.1
Chrysaora hysoscella	21	15	240	-18.9	0.3	14.2	0.3	3.0	0.1	3.6	0.1
Ciliata mustela	5	24	38	-19.3	0.3	16.1	0.2	3.6	0.1	3.4	0.0
Clupea harengus	30	23	139	-19.1	0.2	15.3	0.2	3.3	0.1	3.4	0.1
Cosmetira pilosella	4	10	16	-19.7	0.1	12.5	0.2	2.5	0.1	4.0	0.1
Cyanea capillata	6	44	75	-18.4	0.3	13.1	0.6	2.7	0.2	3.6	0.1
Cyanea lamarckii	18	29	157	-17.4	0.3	13.7	0.3	2.9	0.1	3.4	0.0
Engraulis encrasicolus	5	33	49	-18.9	0.2	15.2	0.2	3.3	0.1	3.2	0.0
Gasterosteus aculeatus	24	22	65	-22.7	0.7	16.9	0.4	3.8	0.1	3.7	0.1
Gastrosaccus spinifer	5	13	15	-17.7	0.3	13.0	0.6	2.7	0.2	3.6	0.0
Hyperia galba	25			-18.1	0.2	14.4	0.3	3.1	0.1	4.0	0.1
Hyperoplus lanceolatus	8	51	149	-19.6	0.2	14.5	0.2	3.1	0.1	3.3	0.0
Idotea linearis	19	13	24	-17.0	0.2	13.1	0.1	2.7	0.0	4.6	0.1
Liparis liparis	5	43	67	-17.5	0.1	15.9	0.2	3.5	0.1	3.3	0.0
Loligo vulgaris	4	15	22	-18.2	0.3	17.8	0.5	4.1	0.1	3.6	0.0
Merlangius merlangus	5	46	120	-18.1	0.3	17.1	0.3	3.9	0.1	3.2	0.0
Mnemiopsis leidyi	74	6	72	-19.0	0.1	14.5	0.2	3.1	0.1	4.0	0.0
Nemopsis bachei	19	7	13	-19.3	0.1	13.8	0.2	2.9	0.1	3.9	0.0
Noctiluca scintillans	1			-20.3		10.3		1.9		6.8	
Osmerus eperlanus	15	52	142	-17.9	0.4	17.2	0.2	3.9	0.1	3.2	0.0
Pleurobrachia pileus	75	7	30	-18.3	0.1	14.7	0.1	3.2	0.0	3.6	0.0
Pleuronectus platessa	5	9	14	-23.1	0.4	15.2	0.9	3.3	0.3	4.0	0.1
Pomatoschistus lozanoi	13	19	58	-18.2	0.3	15.9	0.2	3.5	0.0	3.3	0.0
Pomatoschistus minutus	15	24	82	-18.5	0.4	16.3	0.1	3.6	0.0	3.2	0.0
Praunus flexuosus	6	18	26	-16.1	0.1	14.5	0.3	3.1	0.1	3.3	0.0
Rhizostoma octopus	19	15	130	-19.8	0.1	13.6	0.2	2.8	0.1	3.5	0.1
Sarsia tubulosa	9	10	11	-20.2	0.1	12.3	0.2	2.4	0.0	3.9	0.0
Sprattus sprattus	25	39	132	-18.6	0.1	14.3	0.2	3.0	0.1	3.4	0.1
Syngnathus rostellatus	19	30	139	-18.7	0.1	15.5	0.3	3.4	0.1	3.5	0.1
Trachurus trachurus	10	11	50	-19.8	0.1	16.1	0.3	3.6	0.1	3.3	0.0
Trisopterus luscus	2	80	135	-17.4	0.5	17.6	0.2	4.0	0.1	3.3	0.0
plankton fraction (>80 µm)	5			-16.8	0.4	10.6	0.5	1.9	0.1	7.1	0.4
plankton fraction (<80 µm)	9			-14.6	1.4	8.2	0.4	1.3	0.1	8.6	0.6

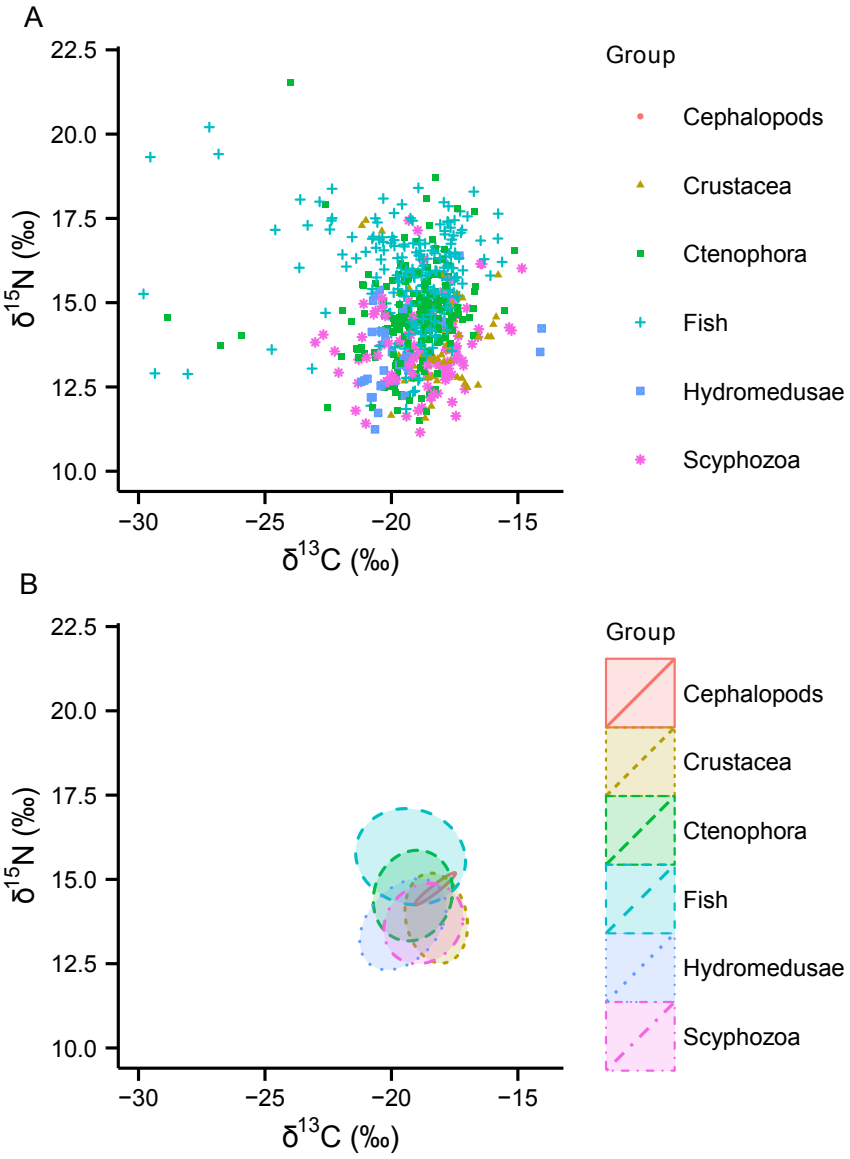

Figure 8.2: (A) δ^{15}N and δ^{13}C values of consumers by taxonomical group and (B) niche of the total group estimated as the Standard Ellipse Area corrected for small sample size (SEAc).

Mnemiopsis leidyi was grouped together in a subcluster with the ctenophore predator *Beroe gracilis* and the sand eel *Hyperoplus lanceolatus*. Cluster B contained the hydromedusa *Aequorea vitrina* and the mysid *Praunus flexuosus*. Cluster C contained several fish species that occupied the highest trophic positions, as well as the the squid *Loligo vulgaris*. Cluster D contains the dinoflagellate *Noctiluca scintillans* and interestingly also two hydroid species, *Sarsia tubulosa* and *Cosmetira pilosella*. Pelagic larvae of plaice *Pleuronectes platessa* were grouped together with the anadromous three-spined stickleback *Gasterosteus acuelatus* in cluster E. Branch F and G consisted solely the different bulk zooplankton fractions.

Seasonal patterns

δ^{15}N and δ^{13}C of most gelatinous zooplankton species varied considerably over the studied period (Fig. 8.4). For ctenophores δ^{15}N was almost constant over the first three months, after which there was an increase for both *Beroe cucumis* and *Pleurobrachia pileus* in June, followed by a decrease in all species in July and August. *B. cucumis* appeared in May and had a 2 ‰ lower δ^{15}N ratio than the other species in this month. Also for *B. gracilis* δ^{15}N ratios were lower than those of its potential prey species *M. leidyi* and *P. pileus* in several months. During the bloom of *M. leidyi* in July and August, when many small ctenophores were present, δ^{15}N of both *Beroe* species decreased at the same time as the δ^{15}N of *M. leidyi* decreased, while the δ^{15}N of *P. pileus* remained constant and ended up being higher than those of the predatory *Beroe* species in August.

Sarsia tubulosa was only caught in March. δ^{15}N of *Aequorea vitrina* increased while that of *Nemopsis bachei* decreased from June to August. *Cosmetira pilosella* was only caught in August.

In March and April only *Cyanea lamarckii* and *Aurelia aurita* were present, which had similar δ^{15}N ratios. The increase in δ^{15}N ratios as observed in the ctenophores in June was also observed in all scyphozoa species except *Rhizostoma octopus* which appeared in June. *R. octopus* and *Chrysaora hysoscella* where the only species present in July and August, and they had similar δ^{15}N ratios in this period.

Patterns in δ^{13}C were less variable in ctenophores. In March *B. gracilis* was depleted compared to its prey. All species of ctenophores showed a similar seasonal pattern, with depletion occurring between June and July, followed by a slight enrichment in August. The hydromedusae *Aequorea vitrina* was the most enriched species and *Cosmetira pilosella* the most depleted one. δ^{13}C values of all species occurring in June– August were different. δ^{13}C of both *A. aurita* and *C. lamarckii* increased from March–May. From June–July both species present showed depletion in δ^{13}C , the highest in *R. octopus*, followed by enrichment as was also observed in the ctenophores.

Isotopic position of *Mnemiopsis leidyi*

The full model (M1) including a different slope for the relationship between δ^{15}N and ctenophore length for each month had the lowest AIC with significant differ-

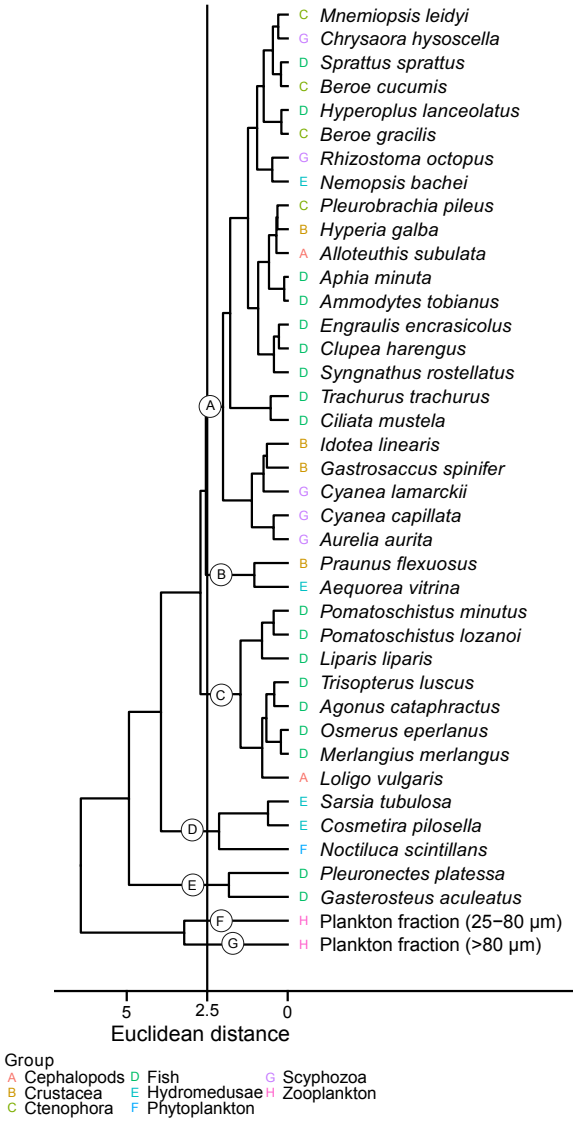

Figure 8.3: Hierarchical cluster analysis of western Wadden Sea pelagic consumers in 2011. Euclidean metric distances calculated with the group-average method based on mean δ^{15}N and δ^{13}C values for each species. Capital letters in circles indicate the main clusters at 2.5 Euclidean metric distance. Coloured letters indicate the taxonomical group to which the species belong.

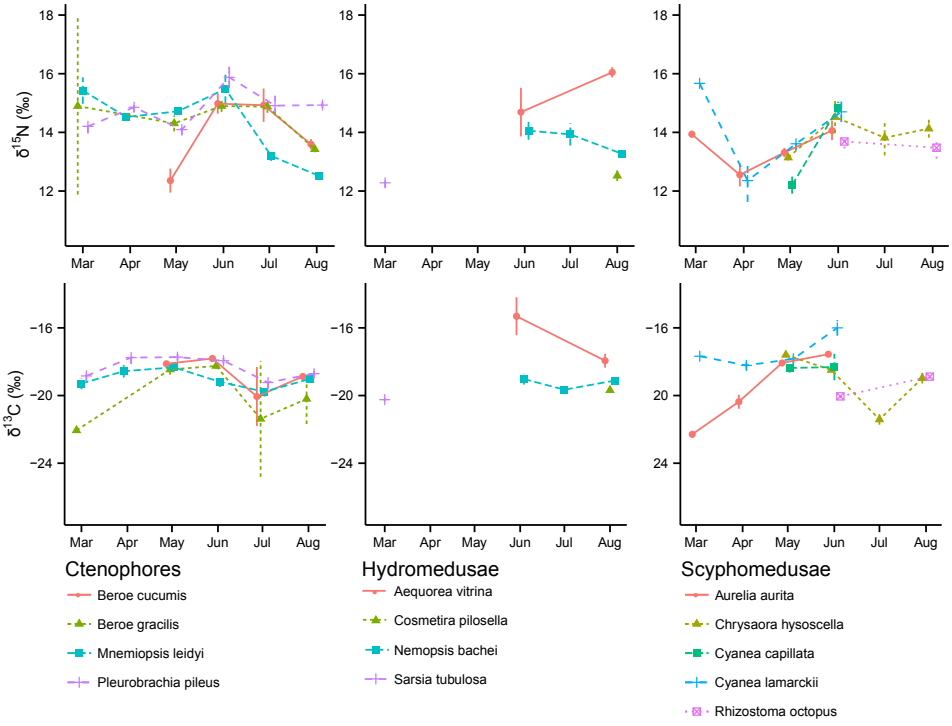

Figure 8.4: average (+/- SE) δ^{15}N per month for all gelatinous species in 2011. Values are slightly dodged along the x-axis.

ences in the interaction between month and length (Table 8.4), so no further model selection was performed. Residuals were normally distributed but a few outliers were present, which all had Cook's Distance values of less than 0.5. There was a significant ($p < 0.05$) relationship between δ^{15}N and ctenophore length, the slope of which differed significantly for all months as illustrated in Figure 8.5, which was partly because not all size classes were present in all months. In general δ^{15}N increased with increasing *M. leidyi* length.

Isotopic niches of species

The size of the Standard Ellipse Area corrected for small sample sizes (SEA_c) gives information on niche width of consumers, whereby the SEA_c of a generalist is larger than that of a specialist feeder. SEA_c was very variable between species as well as within species. The SEA_c of several pelagic consumers overlapped with that of *Mnemiopsis leidyi* in one or more months (Table 8.5). The decrease in δ^{15}N of *M. leidyi* over time and with decreasing length meant that isotopic niche overlap of *M. leidyi* with other species was not constant over the whole studied period but differed each month. SEA_c overlap was highest in the spring months, when the SEA_c of *M. leidyi* overlapped with that of several pelagic fish species, ctenophores,

Table 8.4: Results (estimate with SE in parentheses) for model M1: δ^{15}N of *Mnemiopsis leidyi* in relation to ctenophore length (mm) and month.

	main terms	interactions	
Intercept	11.05^{***}		
	(1.37)		
length	0.12^{**}		
	(0.04)		
April	3.60	length:April	-0.12^{*}
	(2.18)		(0.05)
May	3.75	length:May	-0.12^{*}
	(2.13)		(0.05)
June	3.32^{*}	length:June	-0.09^{*}
	(1.57)		(0.04)
July	1.59	length:July	-0.10^{*}
	(1.11)		(0.05)
August	1.30	length:August	-0.11^{*}
	(0.97)		(0.05)
R^2	0.57		
Adj. R^2	0.49		
Num. obs.	74		

$^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$

scyphomedusae, hydromedusae and cephalopods.

Fish species which had high percentages of SEA$_c$ overlap with *Mnemiopsis leidyi* were the glass goby *Aphia minuta*, the five-beard rockling *Ciliata mustela*,the herring *Clupea harengus*, the sand eel *Hyperoplus lanceolatus*, the lesser pipefish *Syngnathus rostellatus*, the sprat *Sprattus sprattus* and the horse mackerel *Trachurus trachurus*. Gelatinous zooplankton species with overlapping SEA$_c$ with *M. leidyi* were mainly *Beroe gracilis*, *Chrysaora hysoscella*, *Pleurobrachia pileus* and *Nemopsis bachei*. In August no overlap in SEA$_c$ of any species with that of *M. leidyi* was observed.

The cluster analysis presented in Fig. 8.3 was also preformed using only data from a single month (not shown). As not all species were caught in each month and stable isotope values varied within species, the cluster tree topology was very different in each month. In March–June *Mnemiopsis leidyi* clustered together with mainly zooplanktivorous fish such as *C. harengus*. In July, when fewer species could be sampled, no different clusters could be discerned at 2.5 Euclidean Metric Distance. In August *M. leidyi* did not cluster together with *C. harengus* any more and occupied a sub-cluster with *Cosmetira pilosella* and *Beroe gracilis*.

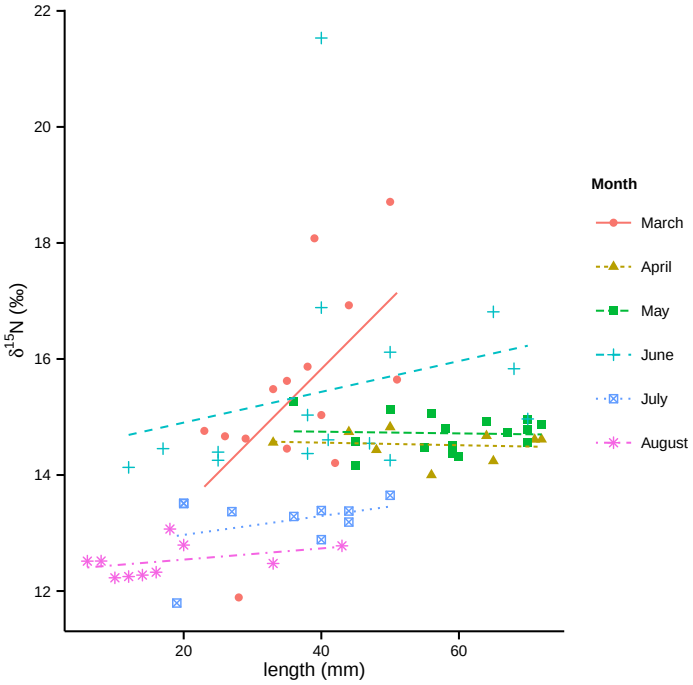

Figure 8.5: Model results for linear model M1 for *Mnemiopsis leidyi* showing the relationship between δ^{15}N and length in different months in 2011, together with observed values.

Table 8.5: Standard Ellipse Area corrected for small sample sizes (SEA_c, as δ units^2) with percent overlap of the SEA_c of consumers with that of *M. leidyi* in parentheses, for each month and for all months combined (overall).

	SEA_c (δ units^2) with percent overlap with *M. leidyi* SEA_c						
Species	March	April	May	June	July	August	overall
Alloteuthis subulata			0.43 (47)				0.43 (51)
Ammodytes tobianus	0.67 (0)	2.22 (9)	0.44 (2)				2.44 (62)
Aphia minuta	3.07 (38)	2.68 (22)	0.76 (42)				2.72 (59)
Aurelia aurita		3.71 (0)	1.45 (0)	1.96 (6)			3.68 (38)
Beroe cucumis			0.52 (0)	1.06 (12)		0.37 (0)	2.19 (51)
Beroe gracilis			1.02 (29)	0.87 (68)		2.34 (0)	4.48 (66)
Chrysaora hysoscella				3.13 (70)		1.97 (0)	3.11 (94)
Ciliata mustela				0.69 (100)			0.69 (41)
Clupea harengus	3 (58)	4.29 (10)	0.24 (4)	3.46 (73)			4.33 (70)
Cyanea lamarckii			1.47 (0)	3.17 (0)			2.57 (5.8)
Engraulis encrasicolus						0.8 (0)	0.80 (99)
Gasterosteus aculeatus	1.99 (0)	3.43 (0)	1.52 (0)	27.89 (0)			17.39 (0)
Gastrosaccus spinifer						3.13 (0)	3.13 (1)
Hyperia galba			2.06 (0)	1.7 (55)			2.55 (35)
Hyperoplus lanceolatus		0.57 (5)					0.57 (100)
Idotea linearis		1.42 (0)	0.56 (0)	0.09 (0)			0.94 (0)
Liparis liparis				0.3 (0)			0.30 (0)
Merlangius merlangus				1.05 (0)			1.05 (0)
Mnemiopsis leidyi	5.52	1.02	0.5	5.81	1.38	0.39	5.21
Nemopsis bachei				2.00 (57)	1.49 (29)	0.25 (0)	1.69 (98)
Osmerus eperlanus			0.25 (0)				0.25(0)
Pleurobrachia pileus	4.34 (29)	2.45 (27)	0.99 (0)	1.61 (24)	3.68 (0)	0.48 (0)	4.09 (64)
Pleuronectes platessa	6.65 (0)						6.65 (0)
Pomatoschistus lozanoi			0.20 (0)				0.20 (85)
Pomatoschistus minutus			3.63 (0)				3.63 (4)
Praunus flexuosus						0.54 (0)	0.54 (0)
Rhizostoma octopus				1.2 (0)			1.20 (30)
Sarsia tubulosa	0.49 (0)						0.49 (0)
Sprattus sprattus		0.93 (0)	1.2 (16)	1.83 (41)		0.29 (0)	1.75 (99)
Syngnathus rostellatus			1.81 (0)	0.9 (2)		0.33 (0)	2.62 (68)
Trachurus trachurus				0.62 (94)		0.63 (0)	1.19 (25)
Overlapping species	3/7	4/9	6/17	12/18	1/2	0/11	24/30

Discussion

The western Wadden Sea receives freshwater and nutrient input from various sources (van Raaphorst and de Jonge, 2004) as well as coastal water input from the nearby North Sea. The average residence time of the water in the Marsdiep tidal basin is ca. 8.5 days (Ridderinkhof *et al.*, 1990). Consequently, species caught in the western Wadden Sea might have spent part of their life feeding in the nearby North Sea, were mesozooplankton δ^{15}N and δ^{13}C are lower (Kürten *et al.*, 2013; Hamer *et al.*, 2011; Frost *et al.*, 2012). For these species our baseline of filter feeding bivalves from within the area might not be appropriate.

The large inputs of anthropogenic nitrogen that enters the western Wadden Sea via several freshwater sources (van Raaphorst and de Jonge, 2004) might also influence the baseline of the system. In Naragansett Bay a clear spatial gradient could be seen in δ^{15}N enrichment of macroalgae, with δ^{15}N becoming more depleted further away from the source, and δ^{15}N of clams collected in the bay being enriched compared to clams collected outside of the bay, suggesting that the clams feed on phytoplankton supported by anthropogenic N (Oczkowski *et al.*, 2008).

Because of this possible variation in baselines between different possible source areas for western Wadden Sea plankton, we always need to consider whether observed variation in δ^{15}N might not be a result of the species feeding on different trophic levels. The addition of a third isotope, sulfur, often helps disentangle sources with similar C and N isotope ratios and might have been useful in this case (Connolly *et al.*, 2004).

Trophic fractionation and Relative Trophic Positions

Trophic fractionation of δ^{13}C ($\Delta\delta^{13}$C) and δ^{15}N ($\Delta\delta^{15}$N) has been shown to be different for different temperatures (Barnes *et al.*, 2007) and diet sources (Caut *et al.*, 2008). In experiments with fish (Barnes *et al.*, 2007) as well as jellyfish (d'Ambra *et al.*, 2014) $\Delta\delta^{15}$N was found to differ from the mean value of 3.4 ‰ used most often for fish (Post, 2002). To our knowledge the trophic fractionation for ctenophores feeding on mesozooplankton and ctenophores feeding on ctenophores has never been studied. For comparison the value of 3.4 ‰ was used in this study. The correction that was applied to δ^{13}C based on the C:N ratio (Post *et al.*, 2007) might not be sufficient enough as this relationship was found to be stronger in *A. aurita* (d'Ambra *et al.*, 2014). A recent meta-analysis has shown that $\Delta\delta^{15}$N is not constant between each trophic level but becomes smaller as sources become more enriched in ^{15}N (Hussey *et al.*, 2014). Not accounting for this leads to an underestimation of the trophic position of an animal with increasing trophic level, especially for trophic level four and higher. As the current study focuses mainly on species around the secondary consumer level and does not include known higher order consumers, this is likely not a big potential bias here.

The species with the highest Relative Trophic Positions were all fish, except for the squid *Loligo vulgaris*. The high RTP of *Aequorea vitrina* likely reflects its nature as a predator of gelatinous zooplankton (Møller and Riisgård, 2007).

Seasonal patterns

While the group of fish species occupied most of the highest trophic positions in this study, a part of their isotopic niche overlaps with that of ctenophores, hydromedusae and scyphomedusae. The cluster analyses show that stable isotopes of many fish species are similar to those of gelatinous zooplankton, which means that all these groups have to be included when assessing the grazing pressure on zooplankton and possible competition for food between fish and other species.

For many species, stable isotope ratios varied over time, which resulted in both the Standard Ellipse Area comparisons as well as the cluster analyses showing large differences between months, suggesting that diet is not constant over time in the Wadden Sea. This has been observed for several fish species in the eastern Wadden Sea using gut content analyses (Kellnreitner *et al.*, 2012).

Interestingly, for several species that appeared in late spring–early summer δ^{15}N, δ^{13}C ratios or both were quite different from those of species that had already been present for several months. The most striking example of this is *B. cucumis* which was depleted in δ^{15}N compared to the other ctenophore species when it appeared in May, followed by enrichment to similar levels in the next month. A possible explanation for this is that the species appeared in the Wadden Sea by advection from the nearby North Sea, where it had been feeding on its preferred prey, the lobate ctenophore *Bolinopsis infundibulum* which has been shown to be depleted in δ^{15}N compared to other species of ctenophores (Hamer *et al.*, 2011). When it entered the Wadden Sea it could have started feeding on the species present there, consequently becoming enriched in δ^{15}N. Something similar might have occurred for *Cyanea capillata* from May–June as well as this is also a species that is known to be more abundant in the North Sea than close to the coast (Hay *et al.*, 1990).

Trophic position of *Mnemiopsis leidyi*

Adult *Mnemiopsis leidyi* can cruise through the water by rhytmic beating of their ciliary comb rows, and generate a feeding current past their tentillae(Colin *et al.*, 2010). This current is difficult to detect by most prey species until they are well between the oral lobes (Colin *et al.*, 2010). Prey items that do not have an escape response to the feeding current are retained directly onto the tentillae, while fast-moving prey such as copepods are caught on the inside surface of the lobes (Waggett and Costello, 1999). Consequently they are a generalist feeder which feeds on a wide range of prey from microplankton to copepods and fish larvae (Cowan and Houde, 1992).

The positive relationship between δ^{15}N and size of *M. leidyi* found in this study suggests that smaller ctenophores have a lower trophic position than larger ones. It has indeed been observed that larval *M. leidyi* are feeding on microplankton (Sullivan and Gifford, 2004, 2007) and that the relative proportion of microplankton in the diet of *M. leidyi* smaller than 10 mm can be above 80% and this proportion becomes smaller with increasing size (Rapoza *et al.*, 2005). Additionally, McNamara *et al.* (2013b) have shown that adult ctenophores decrease competition for their

larvae by feeding on microplankton-grazing mesozooplankton.

The isotopic niche of *M. leidyi* overlapped with those of several fish species, including the herring *Clupea harengus*. A similar overlap in diet between *C. harengus* and *M. leidyi* was found earlier in the eastern Wadden Sea (Kellnreitner *et al.*, 2013). A study by the same authors investigated feeding guild structure of fish species in this area using gut content analysis (Kellnreitner *et al.*, 2012). The division of the fish species in this study is largely in agreement with a similar cluster analysis performed by Kellnreitner *et al.* (2012), with the fish species clustering in cluster C in this study belonging to a guild of mostly *Crangon crangon* eaters, and the species in cluster A belonging to a guild feeding mainly on calanoid copepods. The fish species that showed the most overlap in diet with *M. leidyi* in this study all belonged to cluster A.

Stable isotope ratios of *Mnemiopsis leidyi* also overlapped with those of other gelatinous zooplankton species. These were mainly *Pleurobrachia pileus*, *Chrysaora hysoscella* and *Nemopsis bachei*, all species belonging to the same cluster A.

A striking observation was the lack of overlap of any consumer with *M. leidyi* in August. This happened at the beginning of the bloom period (see Chapters 4 and 5) when almost the entire population consisted of larvae and juveniles. During the bloom period *M. leidyi* is thus using a niche not occupied by any native zooplanktivorous species sampled in this study. The period of high diet overlap with other consumers is also the period in which *M. leidyi* is least abundant, suggesting that *M. leidyi* is not a significant competitor for food for other pelagic gelatinous zooplankton and fish species at present.

The native ctenophore *Beroe gracilis* has been shown to be able to prey successfully on *M. leidyi* in experiments but *M. leidyi* of 20 mm oral-aboral length or larger could only be consumed partially by *B. gracilis* (Hosia *et al.*, 2011). In this study the δ^{15}N of *Beroe* species was often lower than that of its main prey, *P. pileus*. After high densities of small (<10 mm) sized *M. leidyi* started to dominate the catches the δ^{15}N of both *Beroe* species decreased suggesting that small *M. leidyi* is their main prey in this period.

Acknowledgements

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Chapter 9

Synthesis

Lodewijk van Walraven

The objective of this dissertation was to qualify and quantify the role of gelatinous zooplankton in Dutch coastal waters in the past, present and near future, with a focus on the invasive ctenophore *Mnemiopsis leidyi*. In this final chapter I will put our results in a bigger perspective, highlight the main findings and give suggestions for future studies.

In the first part, we reviewed the currently available knowledge on gelatinous zooplankton in the area. We performed additional sampling campaigns in the western Wadden Sea area and analysed available long-term time series. The introduction of the invasive ctenophore *Mnemiopsis leidyi* was the most important change in the gelatinous zooplankton community. This species and its impact on the pelagic ecosystem of Dutch coastal waters were thus the main focus of the second part of the manuscript.

The preceding chapters adressed the following research questions:

- What is the present spatial and temporal distribution of gelatinous zooplankton species in Dutch coastal waters?
- How are these influenced by the many environmental changes observed in the area in the past?
- What are the bottom-up and top-down controlling mechanisms of gelatinous zooplankton in Dutch coastal waters?
- What is the grazing pressure on the zooplankton community, and is there much competition with fish?
- How will projected climatic and other anthropogenically induced changes influence gelatinous zooplankton populations and their importance in Dutch coastal waters?

Main findings

We show that changes have occurred in gelatinous zooplankton species composition and seasonal patterns in Dutch coastal waters (Chapter 2, 4 and 5). The Scyphomedusae have decreased in abundance, but this could not be linked to changes in environmental conditions because of large variation in catches. Seasonal patterns of some species are shifting to an earlier appearance or later disappearance as seasonal water temperatures increase. Scyphozoan polyps found in nearshore and offshore areas all belonged to *Aurelia aurita* and showed population subdivision. The location of the other species' polyps remains unknown.

The overall importance of gelatinous zooplankton as predators has increased because of the introduction of *Mnemiopsis leidyi*. This species is now the most abundant gelatinous zooplankton species in nearshore and inshore Dutch coastal waters in summer and autumn. The year-round presence of *M. leidyi* in Dutch coastal waters, as well as the pattern of dispersion towards other north-western European areas suggests that Dutch coastal waters are a major source region for *M. leidyi* in north-western Europe. The finding of a genotype of *M. leidyi* that can

spawn at low salinity levels suggests that *M. leidyi* from Dutch waters can spread to a wider range of environments than assumed.

Changes in seasonal seawater temperature will likely change the phenology of most gelatinous zooplankton species, with increasing winter and spring temperatures leading to an earlier appearance and possibly bloom formation of Scyphozoa and *Mnemiopsis leidyi*. The increased overlap of the potential spawning window of *M. leidyi* with the zooplankton spring bloom could lead to a major increase in *M. leidyi* bloom magnitude and frequency and thus grazing pressure of the species on the zooplankton.

Long term trends

One limitation of the time series on jellyfish catches in the NIOZ kom-fyke that we analyse in Chapter 2 is that it is only a single station. A comparison of abundance trends in kom-fyke and ANEMOON foundation beach surveys (Fig. 9.1) shows that overall trends are similar and that also in the beach surveys there is a lot of variation between years. In both series *Aurelia aurita* is the most abundant species, followed by *Rhizostoma octopus*, *Chrysaora hysoscella* and *Cyanea* sp. A decrease in *A. aurita* abundance is seen in both series. *C. hysoscella* seems to be increasingly found in the beach surveys in recent decades but this trend is not seen in the kom-fyke data. There also appear to be several years where *C. hysoscella* was absent in the kom-fyke but present in the beach surveys. *Cyanea* sp. abundances in the beach surveys seem to be less variable in the most recent decade than in the years before but no clear trend is visible, similar to the kom-fyke surveys. *R. octopus* abundance shows a decrease in both time series. It seems thus that the general trends observed in the kom-fyke catches are also seen in the beach surveys of stranded jellyfish along the Dutch coast, but the absence of a species in the kom-fyke catches does not mean it was not present along Dutch shores in that year. The most common species of scyphomedusae, *Aurelia aurita* and *Rhizostoma octopus* have decreased in abundance in both time series.

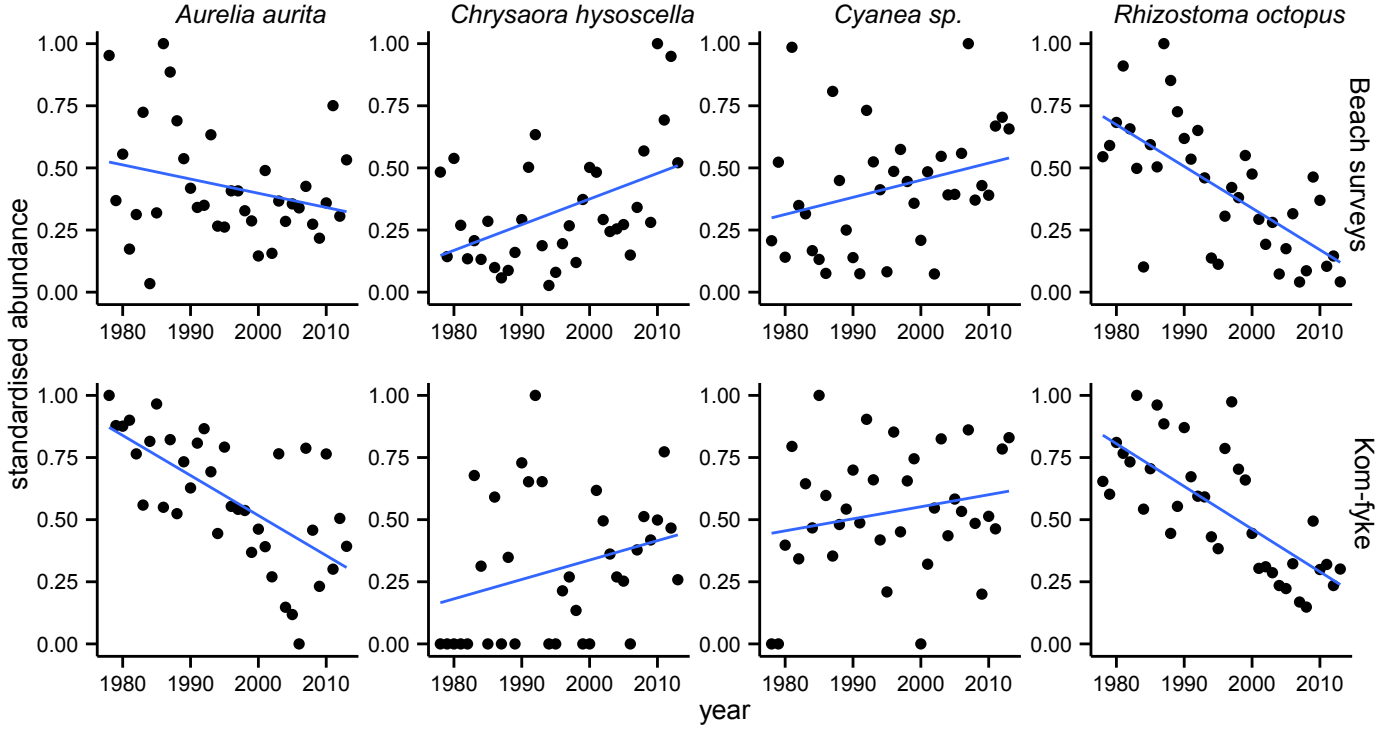

Figure 9.1: Standardised (N/N_{max}) mean abundance of scyphomedusae in ANEMOON foundation beach-combing surveys at eight stations along the Dutch coast 1978–2013 (top row) and in NIOZ kom-fyke catches (bottom row). Abundance in the beach-comb surveys is scored in abundance classes (0, 1-9, 10-99, 100-999), so for comparison the average daily catches in the kom-fyke were log- transformed (base 10). Beach survey data is used with permission of the ANEMOON foundation.

No continuous long-term time series for gelatinous zooplankton other than scyphomedusae existed in Dutch coastal waters. Gelatinous zooplankton species composition, seasonal patterns and abundance in the Wadden Sea was studied in the early 1980s. For this thesis the 1980s sampling programme was repeated in 2009, initially for a single year and focused on *Mnemiopsis leidyi* (Chapter 4) but later extended to 2012 (Chapter 5). Compared to the 1980s, the abundance of most native gelatinous zooplankton species either showed no clear trend or had decreased.

Importance of gelatinous zooplankton taxa as predators of zooplankton

Scyphomedusae are the most conspicuous gelatinous zooplankters found in coastal waters, but how important are they as predators of zooplankton? In Fig. 9.1, Chapter 2 and Chapter 5 we show that the abundance of the main species of scyphomedusae, *Aurelia aurita* and *Rhizostoma octopus*, has decreased. The zooplankton clearance rates estimated for the most common species of Scyphomedusa, *Aurelia aurita*, in the western Wadden Sea were several orders of magnitude lower than those of *Mnemiopsis leidyi* and often also of *Pleurobrachia pileus* (Fig. 5.6). Scyphomedusae and hydromedusae are thus of minor importance as predators compared to ctenophores. *M. leidyi* is thus by far the most important species of gelatinous zooplankton in Dutch coastal waters, which is why the rest of this thesis focused mainly on this species.

Impact of *Mnemiopsis leidyi* on ecosystems in Dutch coastal and inshore waters: present and future.

Predation by *Mnemiopsis leidyi*

Impact on holoplankton

As described in Chapter 5 the introduction of *M. leidyi* has lead to an increase in grazing pressure of gelatinous zooplankton compared to the 1980s, especially in late summer and autumn, a period when in the 1980s grazing pressure by gelatinous zooplankton was very low. This means that, rather than outcompeting local species, it appears that *M. leidyi* has found an empty or under-utilised niche in the Wadden Sea pelagic ecosystem.

Mnemiopsis leidyi densities and clearance rates estimated for *Mnemiopsis leidyi* in the western Wadden Sea in this thesis are some of the highest among invaded areas in north-western Europe, similar to those observed in Limfjorden Riisgård *et al.* (2012) and the eastern Wadden Sea Kellnreitner *et al.* (2013). Clearance rate estimates in this study are likely conservative, as mentioned by Purcell (2009); Riisgård *et al.* (2012) who used the same parameters. Several studies report higher clearance rates (Colin *et al.*, 2010; Granhag *et al.*, 2011). Actual grazing pressure of *Mnemiopsis leidyi* on zooplankton in the area might thus be higher.

Impact on bivalve larvae

In its native range, unlike scyphomedusae (Purcell, 1991a), *Mnemiopsis leidyi* is a known predator of bivalve larvae and can at peak densities remove an average of 20–89 % of bivalve larvae from the water column (McNamara *et al.*, 2010). In invaded areas, *M. leidyi* has been shown to feed on bivalve larvae as well (Granhag *et al.*, 2011). It is thus interesting to see whether bivalve larvae are consumed by *M. leidyi* in the Wadden Sea as well. D-stage bivalve larvae cannot be identified to species level morphologically and to investigate species composition and seasonal patterns of bivalves in the plankton, species specific primers are used (Philippart *et al.*, 2014). We developed a stomach content extraction method and combined this with molecular identification of bivalve larvae in extruded stomach contents (Box 5) to show that *M. leidyi* does indeed consume bivalve larvae in the Wadden Sea.

If clearance rates on bivalve larvae are comparable to those for copepods, *M. leidyi* grazing pressure on bivalve larvae would be significant during the periods of highest grazing rates in August–October (Fig. 5.6). The impact of ctenophore predation on bivalve larvae settlement will differ for different bivalve species, as each has a different spawning period (Philippart *et al.*, 2014). *Macoma balthica*, *Cerastoderma edule*, *Mya arenaria* and *Mytilus edulis* peak spawning happens several months before the *M. leidyi* densities start to increase but peak spawning of *Crassostrea gigas* and *Ensis directus* overlaps with the period of highest *M. leidyi* grazing rates. Interestingly, these species are also invasive in the area.

Right now the highest densities and thus clearance rates of *Mnemiopsis leidyi* take place in summer and autumn. However, as discussed above, increasing temperatures could lead to earlier reproduction and bloom formation of *M. leidyi*, increasing the overlap with peak spawning of bivalves and with the general peak in mesozooplankton food availability (Fransz *et al.*, 1991). The exceptional year of 2009 with blooms of *M. leidyi* already in June (Chapter 4) shows that this is indeed possible.

Top-down control of *Mnemiopsis leidyi* by predation?

Escape from natural predators, the "enemy release hypothesis" is a frequently given reason for why invasive species can rapidly increase in distribution and abundance in invaded areas (Keane and Crawley, 2002). It is not applicable to all invasions (Jeschke *et al.*, 2012). When investigating whether enemy release contributed to the success and extent of an invasion, three arguments have to be considered (Keane and Crawley, 2002). Firstly, top-down control by predators is has to be an important regulating factor for the population. Secondly, predators need to have a greater impact on native than on invasive populations and thirdly, the invaders have to be able to increase their abundance and distribution in response to relaxed predation.

In the native range of the species, *Mnemiopsis leidyi* is predated upon by several predators (Purcell *et al.*, 2001; Arai, 2005; Costello *et al.*, 2012), the majority of which are also gelatinous animals (so-called "intraguild predation", Purcell, 1991b;

Costello *et al.*, 2012). The main predators of *M. leidyi* along the Atlantic coast of the US are the Scyphomedusa *Chrysaora quenquecirrha* (Purcell and Cowan Jr, 1995; Purcell *et al.*, 2001) and the ctenophore *Beroe ovata* sensu Mayer (Kremer and Nixon, 1976; Purcell and Arai, 2001). In the Chesapeake Bay area abundances of *C. quenquecirrha* and *M. leidyi* are often inversely related, suggesting predatory control of the ctenophores by the medusae (reviewed in Purcell *et al.*, 2001) and predation rates of *C. quenquecirrha* on *M. leidyi* can exceed the population growth potential of *M. leidyi* (Condon and Steinberg, 2008; Costello *et al.*, 2012). Similar predatory control may occur by the ctenophore *Beroe ovata*, but since it is less tolerant of lower salinities than *M. leidyi* the latter is sheltered from predation in the more shoreward, low saline areas (Purcell *et al.*, 2001).

In western Europe, *Mnemiopsis leidyi* is predated upon by native species of *Scyphomedusae* (*Cyanea capillata*, Hosia and Titelman, 2011) and ctenophores (*Beroe gracilis*, Hosia *et al.*, 2011). *B. gracilis* is smaller in size relative to *M. leidyi* but it can consume parts of larger prey by ingesting and biting off parts of the lobes of *M. leidyi* (Fig. 1.2). These predators occur in Dutch waters as well, along with other Scyphomedusae and *Beroe cucumis* (Chapter 5). In Chapter 5 we show that densities of native *Beroe* ctenophores did not show a clear increase after the introduction of *M. leidyi*. This suggests that predation by *Beroe* species is at present not controlling the *M. leidyi* population. As *Chrysaora hysoscella* is such an important predator of *Mnemiopsis leidyi* in the native range, it is interesting to see whether the native *Chrysaora* species in Dutch coastal waters, *Chrysaora hysoscella*, could control the *M. leidyi* population in a similar way. The cnidome (types of nematocysts that the species possesses) is similar for both species (Morandini and Marques, 2010) suggesting similar diets.

In Chapter 2 we show that last occurrence of *Chrysaora hysoscella* was related to summer seawater temperature and that the species is present longer in recent decades as average seawater temperatures have increased. Fig. 2.2 shows an increase in *C. hysoscella* catches in summer and autumn in the kom-fyke. In Fig. 9.1 yearly average *C. hysoscella* abundance in beach surveys seems to be increasing as well. The seasonal pattern of *M. leidyi* and *C. hysoscella* overlaps as well (see Fig. 5.4 in Chapter 5) so it is possible that the scyphomedusa is predating on the ctenophore but based on δ^{15}N stable isotope analysis in Chapter 8 the trophic level of both species is almost equal and δ^{15}N and δ^{13}C ratios are mainly overlapping, suggesting that both species have similar diets.

In the Black Sea area *Beroe ovata* appeared more than a decade after the *Mnemiopsis leidyi* invasion, and *M. leidyi* biomass decreased after its introduction (Finenko *et al.*, 2003; Shiganova *et al.*, 2003), suggesting that predation by *Beroe ovata* is controlling the *M. leidyi* population to some extent. Where gelatinous predators are absent, such as in the Caspian Sea, the introduction of *M. leidyi* did have a bigger impact on the ecosystem (Roohi *et al.*, 2008, 2010). In this area a possible deliberate introduction of *B. ovata* was considered (Stone, 2005). Species identification of *Beroe* species is difficult and was found impossible for smaller specimens (Appendix B). As of 2015 *Beroe ovata* has only been observed at a single location in north-western Europe, in the Great Belt, Denmark (Shiganova *et al.*, 2014). *Beroe cucumis* specimens caught in the sampling performed for this

thesis were frequently examined for the presence of anastomosing diverticulae, the distinguishing feature of *B. ovata*, but these were never observed.

Considering the low density of native predators, the lack of an increase in *Beroe gracilis* densities following the *Mnemiopsis leidyi* introduction and the absence of *Beroe ovata* we consider the current potential for top-down control of *M. leidyi* populations in Dutch coastal waters to be low.

Parasites of *Mnemiopsis leidyi*

Aside from predator release, the lack of parasites in invasive populations can also contribute to the abundance and spread of invaders (Torchin *et al.*, 2003).

The most conspicuous parasite of *Mnemiopsis leidyi* is the endoparasitic larva of the sea anemone *Edwardsiella lineata*. Infection by the parasite causes decreases in growth and fecundity. In the native range of *M. leidyi* prevalence of *E. lineata* can be very high and it has been suggested that the decline of the *M. leidyi* population in US waters in fall can be partly attributed to *E. lineata* infection (Bumann and Puls, 1996). While the parasite has been found in *M. leidyi* off the Swedish west coast in 2007 (Selander *et al.*, 2010), in five years of sampling (2009–2013) we have never observed *Edwardsiella* parasites in *M. leidyi* in Dutch coastal waters, making top-down control of the *M. leidyi* population in the area by these parasites currently unlikely.

Dutch estuaries as a source for *Mnemiopsis leidyi* in Europe

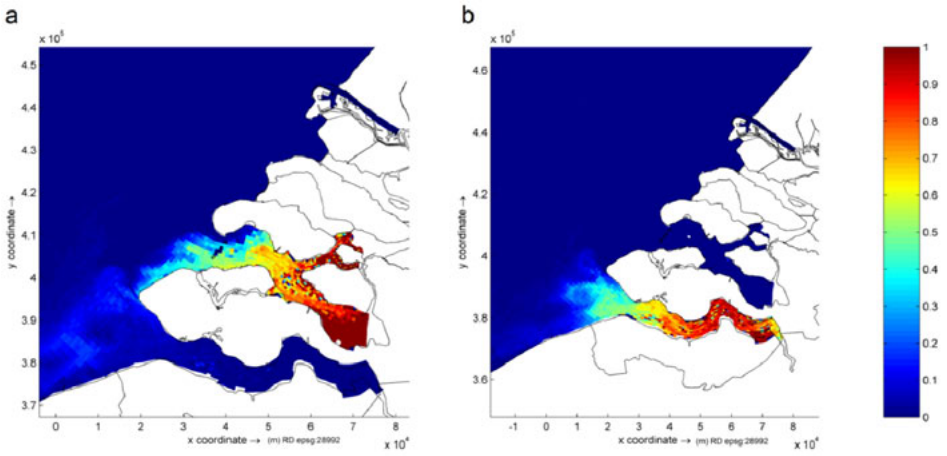

Figure 9.2: (a) Final concentration of particles (N m^{-3}) relative to an assumed initial concentration of 1.0 (N m^{-3}) for the eastern Scheldt July simulation, Delft model; (b) similar for the western Scheldt. Reproduced from van der Molen *et al.* (2015).

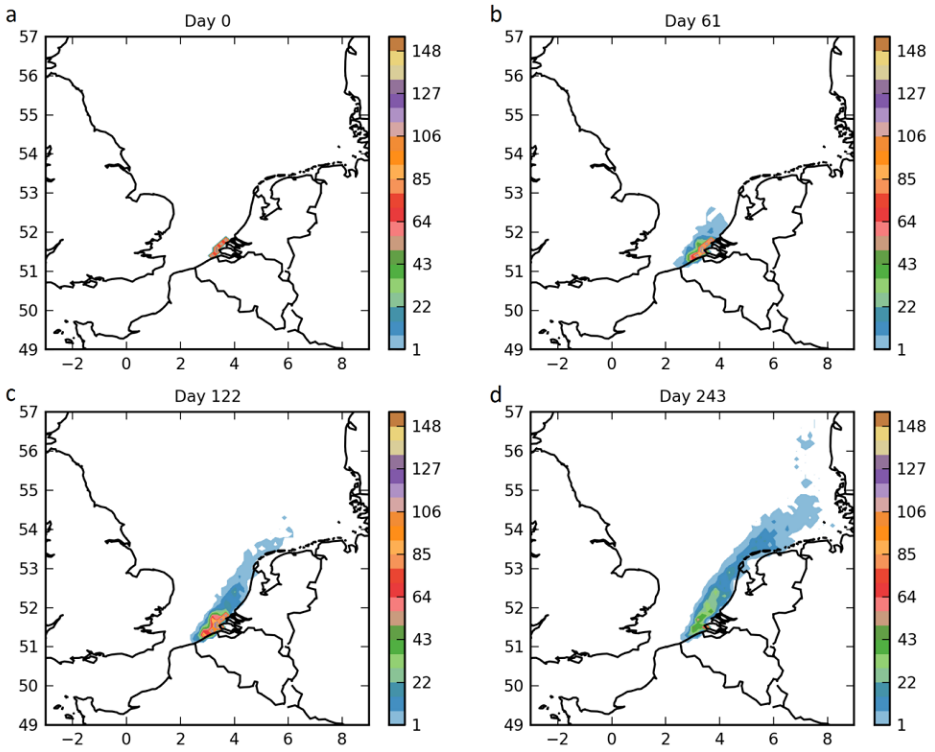

Figure 9.3: Density of particles on the model grid (number of particles per grid cell). (a) on day 1 of the simulation (1 June 2008); (b) on day 61 (31 July 2008); (c) on day 121 (29 September 2008); (d) on day 240 (25 January 2009). Reproduced from van der Molen *et al.* (2015).

In van der Molen *et al.* (2015) we investigated dispersal of *Mnemiopsis leidyi* from the Dutch Delta area to the North Sea using two different hydrodynamic models. Dispersal from the Eastern Scheldt and Western Scheldt areas was investigated using a high resolution 3-D particle tracking model: the Delft3D model. A homogeneous particle distribution as well as a particle distribution based on in-situ observations of ctenophore density in September 2012 were used as starting conditions. When the model was ran for three months, a clear pattern of retention in the back of the estuaries could be seen (Fig. 9.2) but part of the particles are also dispersed into the North Sea. This means that there is enough retention in the area to retain an overwintering population.

Long-distance transport from this area was investigated with a lower resolution model for the North Sea area, the GETM–ERSEM–BFM (3-D General Estuarine Transport Model–European Regional Seas Ecosystem Model–Biochemical Flux Model) with particle tracking (General Individuals Transport Model – GITM). The GETM-ERSEM model reproduction model coupled to a biogeochemical model

modeled survival and reproduction of particles using *Mnemiopsis leidyi* physiological parameters from literature data, and the GITM model modeled the transport of the particles. The simulation was started on 31 July 2008 and ran for 240 days to simulate transport from the bloom period over the winter. This simulation showed that the majority of particles were transported to the north-east as far as the west coast of Denmark. This matches very well with observations of *M. leidyi* in the North Sea in winter described in David *et al.* (2015). The simulation suggests that the Dutch estuaries are a source of *M. leidyi* for coastal areas to the north-east such as the Wadden Sea, German Bight, Limfjorden, Skagerrak and Kattegat. It is likely that the same holds true for the Wadden Sea as well, as in Chapter 4 and 5 we show that *M. leidyi* is present year-round in this area.

Dutch coastal waters are likely not the sole source for *Mnemiopsis leidyi* in areas to the northeast, as the species is observed in more southern locations as well: the Belgian North, several Belgian ports (Van Ginderdeuren *et al.*, 2012; Vansteenbrugge *et al.*, 2015a,b) and also in the French part of the eastern English Channel and the ports of Le Havre (Antajan *et al.*, 2014).

Habitat suitability of the open North Sea area for *M. leidyi* survival and reproduction was investigated in two concurrent studies (Collingridge *et al.*, 2014; David *et al.*, 2015). Both these studies predict highest survival and abundances in nearshore areas, in the German Bight, west of Denmark and Collingridge *et al.* (2014) also in Skagerrak and Kattegat.

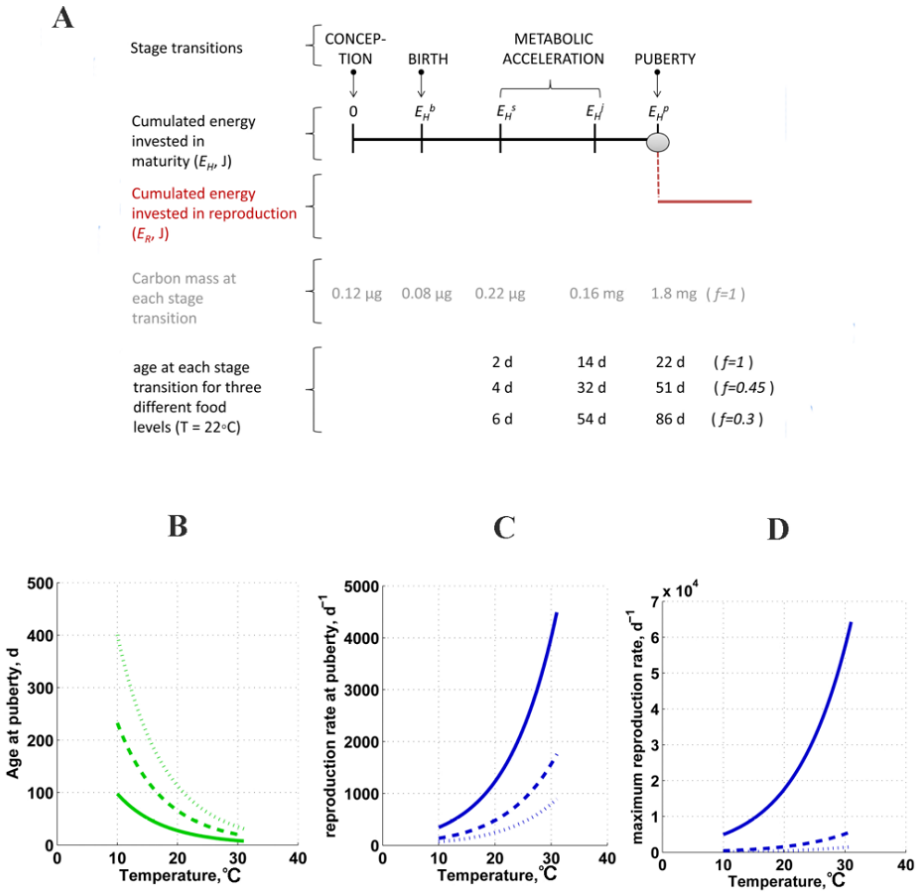

Figure 9.4: Results of DEB model simulations. (a) grey: carbon mass at stage transitions at f = 1. Below are presented the ages at each stage transition for three different ingestion levels ranging from 1 to 0.3. (b) Age at puberty as function of temperature. (c–d) Reproduction rate at puberty and at ultimate mass respectively as function of temperature. (a–d) Simulations are for three ingestion levels: f = 1 (solid line), f = 0.45 (dashed line) and f = 0.3 (dotted line). Reproduced from van der Molen *et al.* (2015).

Survival, growth and reproduction of *M. leidyi* in the southern North Sea area

The role of food and temperature

In van der Molen *et al.* (2015) the Dynamic Energy Budget (DEB) model that was parametrised in Chapter 6 was used to predict survival and reproduction of both adult and juvenile *Mnemiopsis leidyi* in the North Sea. Using the food density and temperature experienced by a particle in the GETM-ERSEM-GITM model simulation (Fig. 9.3) it is shown that larger ctenophores are more sensitive to decreases in food availability than smaller ctenophores but produce much more eggs under favourable food conditions. This relationship is also clear when the DEB model is used to predict age at stage transition and reproductive rates as a function of water temperature for three different food levels (Fig. 9.4). At lower temperatures and lower food levels the predicted age at puberty (the moment allocation of energy to maturation stops and allocation to reproduction starts) is greatly increased. This could be a survival strategy for *M. leidyi* used to survive periods of low food availability. This delay in stage transitions at lower temperatures and food levels has not yet been documented experimentally or in the field and would be an interesting topic for future studies.

In the native range of the species, the timing of *Mnemiopsis leidyi* blooms is changing as a result of warmer winter and/or spring temperature conditions (Condon and Steinberg, 2008; McNamara *et al.*, 2010; Robinson and Graham, 2014); blooms are occurring earlier at increased temperatures. Water temperatures in Dutch coastal waters have increased in the last decades (Van Aken, 2010) and if this increase continues, the phenology of *M. leidyi* will likely shift to earlier blooms in the year.

The role of salinity

Models can be used to predict growth, reproduction, survival and risk of invasion for invasive species in new areas. These often use data from several different sources and populations of origin to estimate model parameters, assuming that the phenotypic response to differing environmental conditions is the same among populations. Examples for *Mnemiopsis leidyi* are Salihoglu *et al.* (2011), Collingridge *et al.* (2014) and Augustine *et al.* (2014a). In Chapter 7 we show that this assumption is not correct for *M. leidyi* as we have found a novel genotype of *M. leidyi* that reproduces at salinities lower than assumed possible in previous studies. This emphasises the importance of validating model assumptions with physiological rate measurements from the populations which are studied since they may show a different environmental tolerance window.

What will be most interesting is how the DEB model parameters (Chapter 6) will differ when they are estimated for low salinities and for different populations. The studies used as source data in Chapter 6 were all performed at saline conditions and as we show in Chapter 7, growth, mortality, reproduction and age and size at puberty are different at a salinity of 8 compared to a salinity of 33. This would also

allow one to investigate which process is actually influenced by salinity: are the maintenance costs increased, the energetic cost per unit of volume or something else? One way of doing this is to treat salinity the same way as toxicants and apply the DEBtox model as described in Jager *et al.* (2006).

In Chapter 7 we introduce the ratio between total length (TL) and oral-aboral length (OA) as a measure for the onset of metamorphosis in *M. leidyi* (Fig. 7.6) and show that this differs for different salinity levels. It would be interesting to compare TL/OA ratios of different *M. leidyi* populations at different salinity levels.

Suggestions for research & management

The missing polyps

In Chapter 3 we present a first survey of distribution and species composition of scyphozoan polyps in the southern North Sea area. Unexpectedly, all polyps found belonged to *Aurelia aurita*. The location of the polyps of the other four species of Scyphomedusae in the area is one of the most interesting questions that still remains. The analysis of polyp population differentiation, where high genetic diversity was found between Dogger Bank samples and other southern North Sea areas, will be interesting to expand to a larger area and to compare genetic differentiation in polyps with those in medusae to be able to link specific medusae aggregations to their polyp origin. Even though in our study only *Aurelia aurita* polyps were found, it cannot be excluded that in previous work polyps of other species were present and it is recommended that any future study on field-sampled polyps includes species identification based either on molecular identification or on the traditional method of rearing, strobilation and identification of ephyrae.

Sampling methodology

Recommendations for sampling, preservation and identification of *Mnemiopsis leidyi* that originated from this thesis are included in Vansteenbrugge (2015). The fixation method using Trichloroacetic Acid that we used for our sampling campaign allows fixation and preservation of *M. leidyi* and storage of samples for longer periods (Appendix A) as well as easier identification of smaller ctenophores (Appendix B). It will be useful when ship-time is limited and detailed measurements of ctenophore length distribution are required. The method could be improved by finding a substitute for the formaldehyde that is still being used in preservation after Trichloroacetic Acid fixation.

Monitoring

What is missing in this thesis is an assessment of zooplankton prey densities before and after the *M. leidyi* introduction. No baseline data on zooplankton density and species composition was available for comparison, and within the scope of this study

it was not possible to do a detailed study of the non-gelatinous mesozooplankton as well.

The EU Water Framework Directive 2000/60/EG stipulates ecological and chemical parameters that EU member states have to monitor in inshore and nearshore waters. The Netherlands has implemented this directive in the "Kaderrichtlijn Water" regulations for monitoring of surface waters. Phytoplankton is included for all water body categories but zooplankton is not (Anonymous, 2014b) and thus there has been very little zooplankton monitoring effort, both qualitatively and quantitatively, in Dutch waters. Every year a monitoring plan for physical, chemical and biological parameters is made, the "Monitoring Waterstaatkundige Toestand des Lands Milieumeetnet Rijkswateren" (MWTL) plan (Anonymous, 2014a). Zooplankton is only monitored in inland, fresh waters but this includes the North Sea canal. The current monitoring protocol only samples surface waters (a bucket sample in waters with currents and a 1.5 m long tube sample for stationary waters) as described in de la Haye (1996) and will thus miss any organisms that are in deeper layers in stratified systems like the North Sea Canal. Sampling volume is 45 L, which might be sufficient for mesozooplankton but will under sample most gelatinous zooplankton species.

As zooplankton are a critical component of pelagic food webs for which monitoring is now lacking in the Netherlands, we argue that yearly monitoring of species composition and density of zooplankton, preferably in every season, should be included in the MWTL programme for fresh, brackish and saline areas. In stratified systems all different water layers should be sampled, at least in the seasons when stratification is occurring. Areas which receive high ballast water loads, such as the ports of Amsterdam and Rotterdam, should receive additional attention.

In the Netherlands, many water bodies were closed off from the sea by man-made barriers and became fresh, the main example being Lake IJssel which was closed off from the Wadden Sea in 1932. In the south of the Netherlands several areas were closed off from the North Sea, one of them being the Lake Grevelingen. After the lake was closed off from the North Sea by the Brouwersdam, water quality issues prompted the construction of a small opening in the Brouwersdam in order to re-salinise the lake (Bannink *et al.*, 1984). Gelatinous zooplankton monitoring in Lake Grevelingen shows *M. leidyi* densities similar to those in the western Wadden Sea (L. van Walraven unpublished data). Several smaller and larger water bodies are or will be re-salinised in the near future. Lake Oostvoorne near the port of Rotterdam was recently re-salinised, and blooms of *M. leidyi* are observed as well (L. van Walraven personal observation). Other semi-enclosed areas, like the Volkerrak-Zoommeer, will likely be good habitat for *M. leidyi* as well when they are re-salinised, and these areas should be monitored for *M. leidyi* presence and abundance.

Appendix

Appendix A

The use of trichloroacetic acid fixation and propylene phenoxetol conservation in quantitative sampling of ctenophores

Lodewijk van Walraven, Victor T. Langenberg, Henk W. van der Veer

This chapter was published as an appendix to Van Walraven *et al.* (2013).

Introduction

Lobate ctenophore species are notoriously difficult to sample due to their fragility and preservation in traditional media such as buffered formaldehyde solution or ethanol is difficult. A method which has largely been unnoticed or possibly considered as too labor intensive (Purcell, 1988) is a method developed for preservation of individual specimens from samples by Adams *et al.* (1976). This method consists of fixation using a trichloroacetic acid (TCA) solution and subsequent preservation in a seawater solution containing propylene phenoxetol, propylene glycol and formaldehyde. In 2009, we tested this method for preservation and fixation of field samples and subsequently applied it with some modifications successfully for a year-round quantitative sampling programme of the ctenophores *Mnemiopsis leidyi*, *Pleurobrachia pileus* and *Beroe gracilis* in the western Wadden Sea (this study). This appendix describes the use of this modified fixation method and its advantages and disadvantages.

Materials and methods

Original fixation and preservation method for single specimens

The fixation and preservation method for individual ctenophores was the result of an investigation of some eighty reagents by the SCOR Working Group 23 project (Adams *et al.*, 1976). For fixation a solution of 1 g tri-chloracetic acid or 1 g p-toluenesulphonic adic in 99 ml sea water is used. For preservation, a solution of 5 ml propylene phenoxetol, 45 ml propylene glycol and 50 ml 40% formaldehyde is prepared. The original protocol includes the following steps:

1. separate the ctenophores from other plankters and place them in a beaker with sea water;
2. drain the sea water away from the ctenophores by using bolting silk;
3. pour the ctenophores gently into the fixative;
4. fix for 30 minutes during which the specimens change slightly from transparency to translucency;
5. remove the fixative and replace it with a 1% preservation/sea water solution;
6. preserve for 5–7 days;
7. transfer from a 1% to a 5% preservation/sea water solution and store between 5 and 20 °C.

Modified protocol for fixation and preservation of quantitative samples

In applying the method of Adams *et al.* (1976) for quantitative samples, the following criteria were used when testing different modifications:

1. fixation and preservation of all species occurred;
2. after shrinkage fresh length and mass could be reconstructed;
3. stomach analysis could be performed on preserved individuals.

These criteria were applied in a three-step procedure: first different concentrations were tested for optimal fixation and preservation. Next, shrinkage and reconstruction of fresh mass was determined and finally, regurgitation was measured.

Ctenophores were caught using the method and gear as described in Van der Veer and Sadée (1984) in tidal gullies in the western Wadden Sea, the Netherlands. The whole sample or a subsample was examined on a 1mm sieve and if possible macroalgae such as *Ulva lactuca* were removed. The sample or a subsample of maximum 500 ml was put in a 800 ml glass jar, which was then filled up with the fixation solution. Solutions were prepared on board by adding different quantities of 100 g/l TCA-seawater solution to ambient seawater. In summer, the fixated samples were stored in a styrofoam box with ice for cooling.

In the lab the TCA-fixed samples were transferred on the day they were caught to sea water containing 1% preservation stock solution by volume. For this, samples were put on and drained in a sieve of ca. 0.5 mm mesh size, returned in the jar and subsequently the preservation solution was added. After 5-8 days the samples were transferred to sea water with 5% preservation stock solution. Samples were stored at a constant temperature of 4 °C.

Ctenophores of 10 mm or smaller in length were measured submerged in a petri dish using a stereo microscope with a measuring eyepiece. Larger ctenophore lengths were measured using a Vernier caliper. For *Pleurobrachia pileus* and *Beroe gracilis* the length measurement taken was the polar length, for *Mnemiopsis leidyi* the distance from the mouth to the statocyst.

Live ctenophores were fixed and preserved individually in glass jars to study shrinkage. Length and weight was measured prior to fixation, when transferred from the 1% to the 5% preservation solution. After preservation length and weight were measured twice with intervals of multiple months. Measurements were taken as described above.

Wet mass was determined by putting individual ctenophores on a drain for 20 sec to remove adhesive water. Ash Free Dry Mass (AFDM) was determined by first drying for 2 to 3 d at 60 °C in a ventilated stove, weighing and incinerating for 2 h at 520 °C. The weight loss after incineration was considered to represent AFDM.

Data analysis

All data were stored in a Microsoft Access database. Data analysis was carried out using R (R Core Team, 2014) and SigmaPlot 12.0. The allometric relationship

$$V = a * l^b \tag{A.1}$$

between the fresh volume (V; ml) and oral–statocyst length before and after fixation (l; mm), was estimated using the non-linear least squares function of R.

Results and discussion

When the TCA solution was added the effect could be observed almost instantly. Structures such as the comb rows, meridional canals, oral lobes and walls of the stomodaeum became increasingly opaque white (Fig. A.3). This property greatly increased the visibility, especially when sorting in black sorting trays.

The fixation and preservation method worked in preserving ctenophores individually as well as together with hydromedusae, crustacean- or ichthyoplankton in a single sample. When samples contained large quantities of plant- peat- or macroalgae fragments or high quantities of crustaceans, often only the mouth, stomodaeum and statocyst complex of the *M. leidyi* ctenophores were left after preservation. Overall ca. 95% of ctenophores could be measured in preserved samples. Large samples of ctenophores smaller than 5 mm length could be subsampled using a Folsom plankton splitter without major damage to the specimens. Of the first 30 *M. leidyi* that were individually fixed, only one could not be measured on the last measuring moment 475 days after fixation in total 7 out of 128 (5.5%) *M. leidyi* disintegrated or were too damaged to measure. One Pleurobrachia pileus had disintegrated and all Beroe gracilis could be measured. Relationships between lengths and weights of fresh and fixed *M. leidyi* are shown in Fig. A.1.

A significant allometric relationship between fixed and fresh lengths and fresh weights of individually fixed ctenophores could be estimated for *M. leidyi* (Fig. A.1; Table A.1) and *P. pileus* (Table A.1). Mean shrinkage by volume as measured when transferring the samples from the 1% to the 5% stock solution was 55% for large *M. leidyi* (n = 8, sd = 4.9) and 54% for small *M. leiydi* (n = 80, sd = 13.1). Percentage of shrinkage was highly variable in small ctenophores.

Mean shrinkage by length was 16% (n = 88, sd = 13.0) post-fixation and 19% (n = 53, sd = 19.8) measured on the second measuring moment. Mean shrinkage by length of fixed *P. pileus* measured on the first measuring moment was 19% (n = 12, st.dev=7.8) and also 19% (n=12, sd = 9.7) measured on the second measuring moment. Mean shrinkage by length of fixed *B. gracilis* after the first and second measuring moment was respectively 26% (n = 20, sdv = 7.0) and 28% (n = 18, sd = 9.1).

There was a positive relationship between ash free dry weight and fresh ctenophore volume (Fig. A.2) for ctenophores weighing less than 20 g ($afdm = 0.0088 * freshweight$). For the ctenophores weighing more than 20 g the sample size was too low to see a clear relationship.

Table A.1: Parameter estimates and standard errors and p-values of the estimated length (fixed or fresh) - fresh volume relationship $V = a * l^b$.

Species	n	estimate	*SE*	*p*
M. leidyi$_{fresh}$	128	a 0.0145	0.0030	<0.001
M. leidyi$_{fresh}$	128	b 2.0000	0.0580	<0.001
M. leidyi$_{fixed}$	128	a 0.0079	0.0013	<0.001
M. leidyi$_{fixed}$	128	b 2.3202	0.0481	<0.001
P. pileus$_{fixed}$	89	a 0.0011	0.0003	<0.001
P. pileus$_{fixed}$	89	b 2.6809	0.1075	<0.001
B. gracilis$_{fixed}$	20	a 0.0022	0.0019	0.273
B. gracilis$_{fixed}$	20	b 2.2282	0.3281	<0.001

No regurgitation was observed in 10 individuals of *M. leidyi* after 10 minutes following addition of the TCA solution to a Petri disk containing the specimen. Food was observed moving towards the mouth opening, but not expelled. After the final preservation step however the stomodaeum wall had turned from translucent to opaque and was often disintegrated into small particles making stomach content analysis difficult or impossible. Only larger crustacean zooplankton prey items such as cyprid larvae and copepods could be distinguished, and in one instance a 6mm long goby larva.

These results show that the method of Adams *et al.* (1976) for the preservation of single species also worked well for the preservation of field samples of ctenophores, allowing detailed quantitative measurements in the lab. Heavily damaged specimens could often still be measured because the mouth-stomodaeum-statocyst complex remained intact (Fig. A.3). This method, while being slightly more labour intensive than the method using Lugol's solution proposed by Engell-Sorensen *et al.* (2009), could potentially be more effective as it does not have the disadvantages of the high variation in shrinkage and discoloration. The percentages of ctenophores that are still measurable are also higher using this method. The Lugol's solution method has been shown to work also for larval ctenophores (Sullivan and Gifford, 2009), whether that is also the case for this method is still unknown. The fixed oral–statocyst length – fresh mass relationship for *M. leidyi* and the other species can be used to accurately estimate the wet mass or bio-volume of live ctenophores. Overall, this method will be very useful for sampling ctenophore populations more efficiently and accurately. A further major improvement to the method would be finding a suitable replacement for the toxic formaldehyde used in preservation.

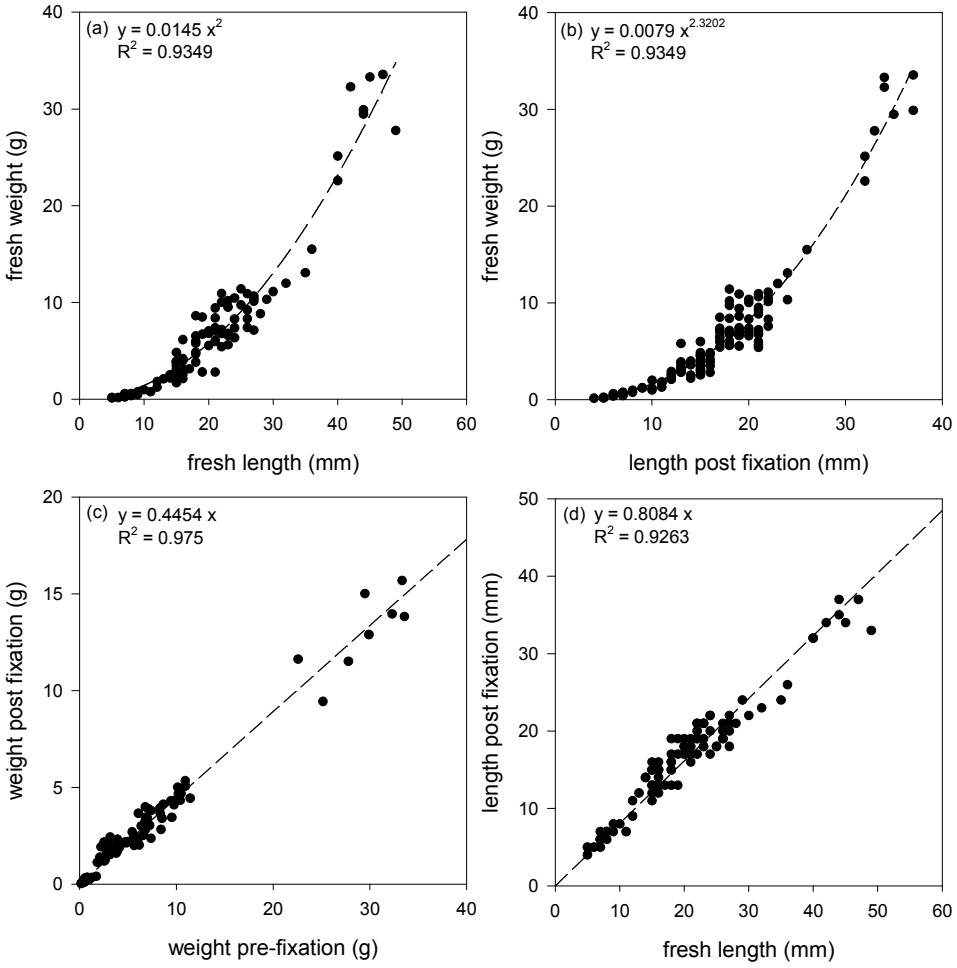

Figure A.1: Relationships between fresh length and fresh weight (a) , fixed length and fresh weight (b), fresh- and fixed weight (c) and fresh and fixed length (d) of individual *Mnemiopsis leidyi*.

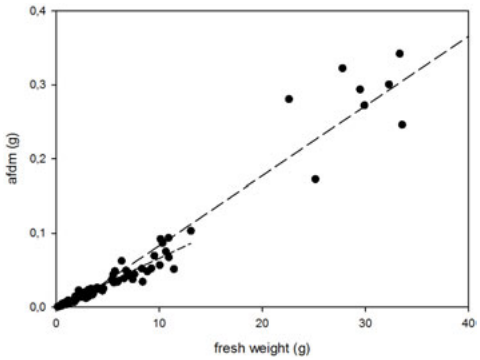

Figure A.2: Relationship between ash free dry weight (afdw, g) and fresh weight of *Mnemiopsis leidyi* ctenophores with fitted regression lines.

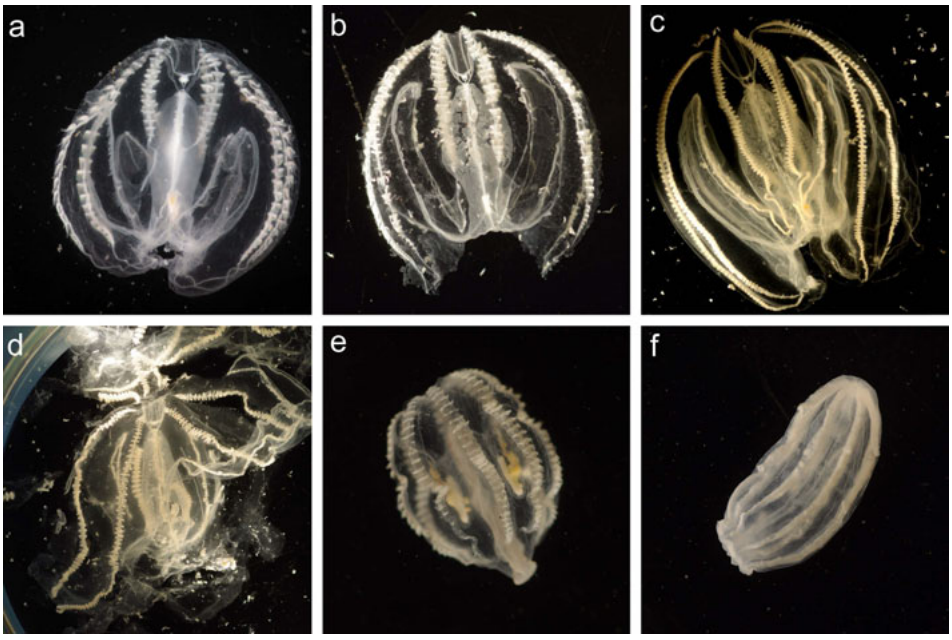

Figure A.3: Photographs of preserved ctenophores: *Mnemiopsis leidyi*: (a) specimen of 8 mm oral–aboral length after 231 days in the preservation solution, (b) specimen of 16 mm oral–aboral length after 475 days in the preservation solution, (c) specimen of 34 mm oral–aboral length after 397 days in the preservation solution, (d) damaged preserved specimen of 32 mm oral-aboral length after 397 days in the preservation solution. Note that the statocyst-stomodaeum-mouth complex is still intact allowing measurement. (e) specimen of *Pleurobrachia pileus* 14 mm length after 397 days in the preservation solution. (f) specimen of *Beroe gracilis* of 16 mm length after 358 days in the preservation solution.

Appendix B

Identification key for ctenophora in Dutch coastal waters

This key can be used for the identification of ctenophores in Dutch coastal waters (excluding overseas territories).

The identification of ctenophores can be very difficult, especially when dealing with damaged specimens. This key for the identification of ctenophores found in Dutch coastal waters is based upon the experience obtained identifying ctenophores in North Sea, Wadden Sea and coastal waters of Zeeland in the period 2009–2014 and the ICES ID leaflet "ctenophora" (Greve, 1975).

Identification is best performed on submerged specimens, e.g. in a petri dish, with oblique lighting from a cold light source or transverse lighting using a light table. A binocular microscope is necessary.

Key

1. (a) Comb rows along the longitudal axis of the body. Bilaterally symmetric. 2

   (b) No comb rows, radially symmetric. **Scypho- or hydromedusa**

2. (a) Larger than 5 mm 3

   (b) Smaller than 5 mm 7

3. (a) Spherical or spheroid. Two long finely branched tentacles.

NL: Zeedruif EN: Sea gooseberry *Pleurobrachia pileus*

(b) Flattened, fragile 4

4. (a) Oval, large mouth occupying the entire width of the animal. 5

(b) Not oval, with two oral lobes. 6

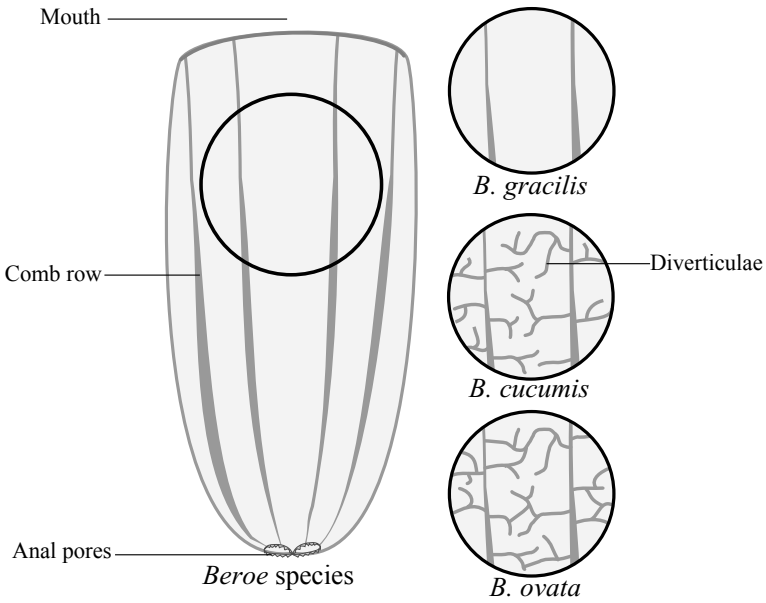

Figure B.1: Features distinguishing the three species of *Beroe* mentioned in this key.

5. *Beroe* species can be distinguished by the presence and characteristics of diverticulae, canals diverging from the meridional canals under the comb rows (Fig. B.1). These do not develop until the animal reaches a length of approximately 20 mm and thus *Beroe* specimens smaller than this can not be identified to species level.

(a) No diverticulae. Maximum length 35 mm.

NL: slanke meloenkwal *Beroe gracilis*

(b) Diverticulae diverging from the canals under the comb rows. The diverticulae of parallel comb rows are never connected. Can be bigger than 35 mm.

NL: grote meloenkwal *Beroe cucumis*

(c) Diverticulae diverging from the canals under the comb rows. Some diverticulae of parallel comb rows are connected (anastomose). Can be bigger than 35mm.

***Beroe ovata* (not yet recorded in Dutch coastal waters)**

6. (a) Oral lobes terminate near or past the statocyst (Fig. B.2).
NL: Amerikaanse ribkwal EN: Sea walnut *Mnemiopsis leidyi*

(b) Oral lobes terminate nearer to the mouth than to the statocyst (Fig. B.2). One comb row on each lobe has a black stripe (often not visible).

Bolinopsis infundibulum

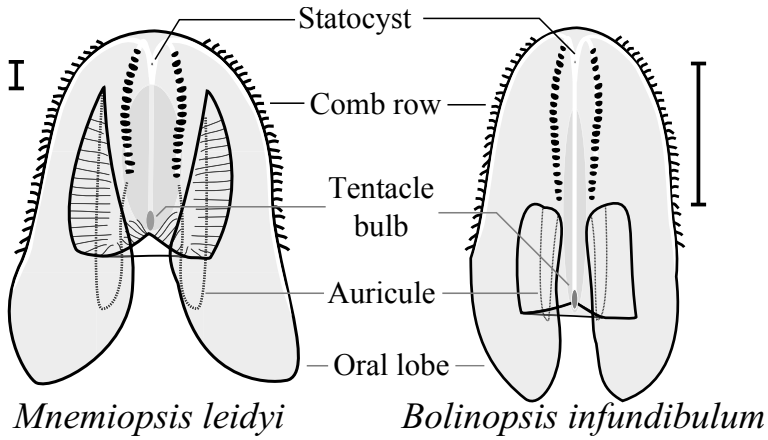

Figure B.2: *Bolinopsis infundibulum* and *Mnemiopsis leidyi* compared, with the distance from the statocyst to the termination of the oral lobe indicated.

7. (a) Tentacle bulbs present. 8

(b) No tentacle bulbs.

Beroe, not identifiable to species level at this size.

8. **Cydippid larvae.** Note: Cydippid stage ctenophores are very difficult to distinguish. The below characteristics are visible using a binocular microscope or similar magnification. Small individuals of the Arctic species *Mertensia ovum* found in the Baltic Sea together with *M. leidyi* are very similar to *M. leidyi* and require genetic indentification (Gorokhova *et al.*, 2009; Gorokhova and Lehtiniemi, 2010). Especially samples of small ctenophores only, without larger specimens in the same sample should be treated with caution. Visibility of the comb rows is much better in TCA-fixed specimens (Fig. B.3) than in fresh specimens.

(a) Rounded tentacle bulbs. Comb rows thinner compared to *Pleurobrachia pileus* in direct comparison (Fig. B.3).
NL: Amerikaanse ribkwal EN: Sea walnut *Mnemiopsis leidyi*

(b) Elongated, non-round tentacle bulbs. Comb rows thicker compared to *Mnemiopsis leidyi* in direct comparison (Fig. B.3).
NL: Zeedruif EN: Sea gooseberry *Pleurobrachia pileus*

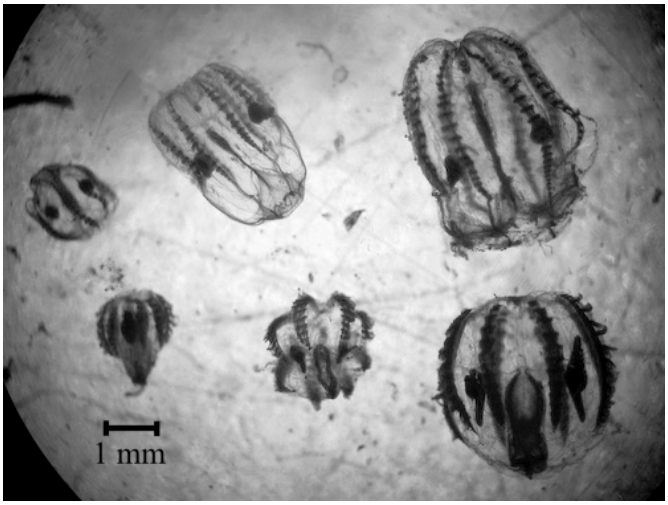

Figure B.3: Comparison of TCA-fixed cydippid stages of *Mnemiopsis leidyi* (top three individuals) and *Pleurobrachia pileus* (bottom three individuals) of different sizes. Viewed through a binocular microscope using transverse lighting.

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STERN

Summary

Gelatinous zooplankton are an understudied group in many areas. The arrival of a notorious invasive species, the ctenophore *Mnemiopsis leidyi* in Dutch coastal waters instigated a series of sampling programmes and triggered a renewed interest in the ecology of this diverse group of animals. The main goal of this thesis is to investigate the present spatial and temporal distribution of gelatinous zooplankton species in Dutch coastal waters. How are these influenced by the many environmental changes observed in the area?

The thesis is divided into two parts: a first part focused on investigating changes in gelatinous zooplankton species composition, seasonal patterns and abundance and a second part focused on gaining insight in the mechanisms that make *M. leidyi* such a successful invasive species.

Questions asked at the start of the study were: What is the present spatial and temporal distribution of gelatinous zooplankton species in Dutch coastal waters? How are these influenced by the many environmental changes observed in the area in the past? What are the bottom-up and top-down controlling mechanisms of gelatinous zooplankton in Dutch coastal waters? What is the grazing pressure on the zooplankton community, and is there much competition with fish? How will projected climatic and other anthropogenically induced changes influence gelatinous zooplankton populations and their importance in Dutch coastal waters?

Gelatinous zooplankton in Dutch coastal waters

As a first step, available studies and data on gelatinous zooplankton in Dutch coastal waters were reviewed. In the Marsdiep area of the western Wadden Sea, the NIOZ Royal Netherlands Institute for Sea Research has operated a kom-fyke fish trap, a type of passive fishing gear. From 1960 onwards the catch of this kom-fyke was recorded each day for the spring, autumn and sometimes summer season. Catches of jellyfish in the kom-fyke were also counted and recorded. This unique 50 year time series was analysed in **Chapter 2** where changes in phenology, abundance and species composition of Scyphozoan jellyfish are related to changing environmental conditions. All species appeared earlier in the year in recent decades, which at least for one species (*Aurelia aurita*) was related to increasing winter temperatures. Abundance trends could not be related to changing environmental conditions because of high variation in the data, which could imply that population

regulating mechanisms operate mainly during the sessile polyp stages.

The location of these sessile polyp stages of Scyphozoa is unknown for many species which is why in **Chapter 3** the distribution, species composition and population structure of jellyfish polyps is investigated by sampling a variety of different natural and artificial substrates in the southern North Sea and identify them using molecular markers. Unfortunately all polyps that were found in nearshore and offshore areas belonged to *Aurelia aurita* and thus the location of the other species' polyps remains unknown. The high number of *A. aurita* polyp samples did allow us to perform the first study on population structure of Scyphozoa based on polyps and not medusae. *A. aurita* polyps showed population subdivision whereby polyps from the central North Sea differed from those in the other areas.

The most recent quantitative sampling programmes focused on gelatinous zooplankton in both the Dutch Wadden Sea as well as the estuaries of Zeeland dated from the 1980s, which is before *Mnemiopsis leidyi* was present. In 2009 the 1980s sampling programme in the Marsdiep area of the western Wadden Sea was repeated using similar methods and stations. In **Chapter 4** we show that nowadays *Mnemiopsis leidyi* is present in high densities in the area. Following this, the western Wadden Sea monitoring programme was continued in 2010–2012 and species composition, seasonal patterns and zooplankton grazing pressure are compared with those in the 1980s in **Chapter 5**. Because of the introduction of *M. leidyi* the overall importance of gelatinous zooplankton as predators has increased. *M. leidyi* is now the most abundant gelatinous zooplankton species in near-shore and inshore Dutch coastal waters. In the 1980s grazing pressure on the zooplankton by gelatinous predators was low in summer and autumn but in 2009–2012 high densities of *M. leidyi* exerted a high grazing pressure on the zooplankton, with a peak in September. This is a major change in the Wadden Sea pelagic ecosystem, which has likely occurred in the estuaries of Zeeland as well. It appears that *M. leidyi* has found an empty or under-utilised niche in the Wadden Sea pelagic ecosystem.

The invasion success of *Mnemiopsis leidyi* investigated

In **Chapter 6** modelling and data analysis is combined to study the energy budget of *M. leidyi* over its full life-cycle using Dynamic Energy Budget (DEB) theory and literature data to investigate the response of different life stages to changes in food and temperature. An analysis of data obtained at temperatures ranging from 8 to 30 °C suggests that the optimum thermal tolerance range of *M. leidyi* is higher than 12 °C. Furthermore *M. leidyi* seems to undergo a so-called metabolic acceleration after hatching. Intriguingly, the onset of the acceleration appears to be delayed and the data do not yet exist which allows determining what actually triggers it. It is hypothesised that this delay confers a lot of metabolic flexibility by controlling generation time.

Although *Mnemiopsis leidyi* can tolerate a broad range of temperatures and salinities in its native range, low salinity limits its range expansion in parts of

northern Europe. Large *M. leidyi* blooms have been observed in the brackish North Sea Canal near Amsterdam in the Netherlands, at salinities considered too low for successful reproduction. In **Chapter 7** the influence of salinity as a factor limiting the spread of *M. leidyi* in invaded areas is studied in a common-garden experiment where *M. leidyi* from the low salinity Amsterdam population and a nearby marine population were acclimatised and raised at two salinity levels. This experiment shows that *M. leidyi* can complete its entire life cycle at a salinity of 8, albeit with much higher mortality than at a salinity of 33. Genotyping of the animals surviving at the end of the experiment revealed high differentiation between sub-populations of origin. Within the Amsterdam sub-population high genetic differentiation was found. This is the first observation of a low salinity genotype of *M. leidyi* in Europe, which could spread to yet uninvaded areas where environmental parameters were previously thought to be limiting.

In **Chapter 8** competition between *Mnemiopsis leidyi* and other zooplanktivorous species in the Wadden Sea is qualified by estimating diet overlap of fish, scyphozoa, hydromedusa, ctenophores, crustaceans and cephalopods of the western Wadden Sea using Stable Isotope Analysis. A cluster analysis showed that average δ^{13}C and δ^{15}N ratios of the invasive *M. leidyi* were similar to those of fish species of intermediate trophic level such as the glass goby *Aphia minuta*, the herring *Clupea harengus* and the horse mackerel *Trachurus trachurus*. Diet overlapped with that of most other gelatinous zooplankton species as well, such as the compass jellyfish *Chrysaora hysoscella*, the sea gooseberry *Pleurobrachia pileus* and the hydroid *Nemopsis bachei*. δ^{15}N of *M. leidyi* was positively related to ctenophore size, suggesting that small ctenophores occupy a lower trophic level than large ones. At the beginning of the bloom period in August when almost the entire population consisted of larvae and juveniles there was no overlap in isotopic niche of *M. leidyi* with that of any other pelagic zooplanktivore. The period of high diet overlap with other consumers is also the period in which *M. leidyi* is least abundant. This suggests that at present, *M. leidyi* is not a significant competitor for other gelatinous zooplankton and fish species. During the bloom period of *M. leidyi* the abundance of competing species is low, suggesting that *M. leidyi* is using an unoccupied niche.

The future of Dutch gelatinous plankton

This thesis increases our understanding of gelatinous zooplankton phenology, abundance and species composition in Dutch coastal waters and provides tools such as the Dynamic Energy Budget model of *Mnemiopsis leidyi*, the application of fixation and preservation of *M. leidyi* in quantitative sampling and a common garden experiment to study the phenotypical response of different *M. leidyi* populations to differing environmental conditions. These tools can be applied in the study of gelatinous zooplankton in other areas as well.

Our study on sampling, identification and population structure of Scyphozoan polyps (**Chapter 3**) would be interesting to expand to a wider area and different habitat types as the location of polyps other than those of *Aurelia aurita* remains

unknown.

In **Chapter 9** an application of the Dynamic Energy Budget (DEB) model parametrised in Chapter 6 is presented. Using ecological modelling we show that *M. leidyi* ctenophores can be transported from the southern North Sea to the central North Sea and German Bight and subsequently, the DEB model predicts survival and reproduction of *M. leidyi* in the conditions experienced along the transport trajectory. This shows that Dutch coastal waters can be a source of *M. leidyi* for other western and northern European waters. The application of the DEB model yields some interesting predictions: larger *M. leidyi* ctenophores are more sensitive to decreases in food availability than smaller ctenophores but produce much more eggs under favourable food conditions, and at lower temperatures and lower food levels the predicted age at puberty (the moment allocation of energy to maturation stops and allocation to reproduction starts) is greatly increased. This could be a survival strategy for *M. leidyi* used to survive periods of low food availability. Both these predictions could be tested experimentally. The experiment of **Chapter 7** shows that reproduction, growth and metamorphosis of *M. leidyi* are different at lower salinity levels. Consequently, DEB parameters will likely also differ.

Concluding, the results in this thesis show that the introduction of *Mnemiopsis leidyi* in Dutch coastal waters has not yet had a large impact on the populations of native zooplanktivorous fish and plankton. However, as *Mnemiopsis leidyi* reproduction and growth is strongly related to temperature, climate induced warming could cause shifts in its phenology and blooms in periods or areas where temperature was previously limiting. Similarly, the finding of a *M. leidyi* genotype able to live at lower salinity levels could lead to blooms in unexpected locations. This means that continued monitoring of *M. leidyi* presence and abundance is important. Unfortunately, monitoring of zooplankton in Dutch coastal waters is lacking in coastal as well as inshore waters and blooms of *M. leidyi* or introductions of other invasive species are currently going unnoticed.

Samenvatting

Kwalachtigen zijn een onderbestudeerde diergroep in veel zeegebieden. In Nederlandse kustwateren was de vondst van de invasieve uitheemse ribkwal *Mnemiopsis leidyi* aanleiding voor de start van een serie nieuwe onderzoeken naar de ecologie van kwalachtigen in het gebied en een hernieuwde interesse in deze diverse diergroep. Het hoofddoel van dit proefschrift is om de huidige verspreiding, het voorkomen en de seizoenspatronen van kwalachtigen in de Nederlandse kustwateren in kaart te brengen. Hoe worden deze beinvloed door de vele veranderingen in de omgeving in dit gebied?

Het proefschrift bestaat uit twee delen: een eerste deel gefocused op het onderzoeken van veranderingen in soortensamenstelling, seizoenspatronen en voorkomen van kwalachtigen en een tweede deel gericht op het verkrijgen van inzicht in de mechanismen die van de ribkwal *Mnemiopsis leidyi* zo'n successvolle invasieve soort maken.

Vragen die aan het begin van het onderzoek werden gesteld waren: Wat is de huidige verspreiding in tijd en ruimte van kwalachtigen in de Nederlandse kustwateren? Hoe zijn deze beïnvloed door veranderingen in de omgeving? Door welke top-down en bottom-up processen worden kwalachtigen beïnvloed? Wat is de predatiedruk op de dierlijke planktongemeenschap en is er veel concurrentie met vissen? En hoe worden populaties van kwalachtigen in Nederlandse kustwateren beïnvloed door klimaatverandering en andere anthropogene invloeden?

Kwalachtigen in Nederlandse kustwateren

Aan het Marsdiep, een zeegat in de westelijke Waddenzee, beheert het NIOZ Koninklijk Nederlands Instituut voor Zeeonderzoek een kom-fuik, een passief vistuig. Sinds 1960 is elke dag de vangst uit de kom-fuik geregistreerd, voornamelijk in het voor- en najaar maar soms ook in de zomer. Aantallen gevangen kwallen per soort in de fuik zijn ook genoteerd. Dit heeft een unieke tijdserie opgeleverd van meer dan vijftig jaar aan kwallenvangsten, die in **hoofdstuk 2** is gebruikt om veranderingen in fenologie, voorkomen en soortensamenstelling van kwallen in relatie tot omgevingsfactoren te onderzoeken. Hier laten we zien dat in de recente decennia alle soorten eerder in het jaar verschenen, wat voor de oorkwal *Aurelia aurita* gerelateerd was aan een toegenomen zeewater temperatuur in de winter. De kompaskwal *Chrysaora hysoscella* blijft ook tot later in het jaar nog aanwezig.

Door de grote dagelijkse variatie in vangsten konden patronen in het voorkomen van kwallen niet gerelateerd worden aan veranderingen in omgevingsvariabelen. Dit zou kunnen impliceren dat populatie-regulerende processen vooral optreden in het poliepstadium van de kwallen. Wel waren de vangsten in de fuik in recente decennia een stuk lager dan in de jaren 80 en 90; een periode die gekenmerkt werd door een hoge mate van eutrofiëring van de Waddenzee.

Door een vestigingsplaats voor kwalpoliepen te bieden kunnen door mensen gebouwde structuren in zee bijdragen aan de vorming van kwallenbloeien. De locatie van de vastzittende poliepstadia van de schijfkwallen was onbekend voor veel soorten, ook in Nederlandse kustwateren. Daarom is in **hoofdstuk 3** de verspreiding, soortensamenstelling en populatie structuur van kwalpoliepen onderzocht door het nemen van monsters van poliepen van verschillende substraten in de zuidelijke Noordzee en aangrenzende gebieden. Omdat poliepen van elke soort er vrijwel identiek uit zien werden de poliepen aan de hand van genetische verschillen op soortniveau geïdentificeerd. Helaas behoorden alle gevonden poliepen tot de oorkwal *Aurelia aurita* waardoor de locatie van de poliepen van de andere soorten nog steeds niet is gevonden. Het hoge aantal *A. aurita* monsters maakte het echter wel mogelijk om de eerste studie aan de populatiestructuur van kwallen die gebaseerd is op poliepen in plaats van medusen uit te voeren. *A. aurita* poliepen vormden verschillende populaties, waarbij poliepen uit de centrale Noordzee een andere populie vormden dan poliepen uit de kustgebieden.

De meest recente kwantitatieve monsterprogramma's gericht op kwalachtigen in zowel het Waddengebied als in Zeeuwse wateren stammen uit de jaren 80, toen *Mnemiopsis leidyi* hier nog niet voor kwam. In 2009 is daarom het in de jaren 80 uitgevoerde monsterprogramma in en rond het Marsdiep herhaald met dezelfde methodes en op dezelfde plek. In **hoofdstuk 4** laten we zien dat *M. leidyi* in hoge dichtheden voorkomt in dit gebied, vooral in de nazomer en het najaar. Dit monsterprogramma is vervolgd in 2010–2012 en in **hoofdstuk 5** wordende soortensamenstelling, seizoenspatronen en predatiedruk op het dierlijk plankton vergeleken met die in de jaren 80. Door de introductie van *M. leidyi* is de rol van kwalachtigen als predatoren van het dierlijk plankton toegenomen. De soortensamenstelling is grotendeels hetzelfde, met één belangrijke uitzondering: *M. leidyi* is nu de meest algemene kwalachtige in Nederlandse kustwateren. In de jaren 80 was de predatiedruk van kwalachtigen op het dierlijke plankton laag, behalve in het voorjaar tijdens de voorjaarspiek van *Pleurobrachia pileus*. In 2009–2012 echter zorgden hoge dichtheden *M. leidyi* voor een hoge predatiedruk op het plankton ook in de zomer en het najaar. Op sommige dagen zou in theorie al het plankton in de waterkolom binnen een dag weggefilterd kunnen worden. Dit is een belangrijke verandering in het ecosysteem van het vrije water in de Waddenzee, die waarschijnlijk in Zeeuwse wateren ook is opgetreden. Het lijkt erop dat *M. leidyi* een on- of ondergebruikte niche heeft gevonden in het ecosysteem als predator van dierlijk plankton in nazomer en herfst.

Het succes van *Mnemiopsis leidyi* als invasieve exoot onderzocht

In **hoofdstuk 6** wordt het energiebudget van *Mnemiopsis leidyi* gedurende de gehele levenscyclus onderzocht met behulp van de Dynamic Energy Budget (DEB) modeltheorie. Aan de hand van gegevens uit de literatuur over parameters als grootte, groei, voortplanting en respiratie is een DEB model geparametriseerd voor *M. leidyi*, wat daarna is gebruikt om de respons van verschillende stadia van *M. leidyi* op verschillen in voedselbeschikbaarheid en temperatuur te onderzoeken. Data verzameld bij temperaturen van 8 tot 30 °C suggereren dat de optimale tolerantiegrens qua temperatuur bij *M. leidyi* hoger ligt dan de 12 °C die eerder werd aangenomen. Verder lijkt *M. leidyi* zogenoemde metabolische acceleratie (een afwijking van het standaard Von Bertalanffy groeimodel) te ondergaan na het uitkomen van het ei. Deze acceleratie lijkt vertraagd te worden door een nog onbekende factor. De modelsimulaties suggereren dat *M. leidyi* door het vertragen of versnellen van de groei en het beïnvloeden van de generatietijd erg veel metabolische flexibiliteit heeft en zich aan kan passen aan zowel lage als hoge voedselbeschikbaarheid.

Alhoewel *Mnemiopsis leidyi* bij een brede range van temperatuur en zoutgehalte kan overleven in zijn oorsprongkelijke leefgebied, lijkt in veel gebieden waar de soort is geïntroduceerd de verspreiding gelimiteerd te zijn bij lage zoutgehaltes. Dit maakte het optreden van hoge dichtheden *M. leidyi* in het brakke Noordzeekanaal bij Amsterdam opmerkelijk, aangezien het zoutgehalte hier veelal te laag zou zijn om voortplanting van *M. leidyi* mogelijk te maken. In **hoofdstuk 7** is de rol van zoutgehalte als limiterende factor voor de verspreiding van *M. leidyi* onderzocht in een “common-garden” experiment waarin ribkwallen afkomstig uit brak water bij Amsterdam en ribkwallen afkomstig uit zout water bij Texel werden geacclimatiseerd en opgekweekt bij twee verschillende zoutgehaltes: 8 en 33. Tijdens het experiment werden overleving, groei en voortplanting dagelijks gemeten. In dit experiment kon *M. leidyi* zijn hele levenscyclus voltooien bij een laag zoutgehalte van 8. Minder ribkwallen overleefden het experiment bij een zoutgehalte van 8 dan bij een zoutgehalte van 33. Genetische analyse van de ribkwallen die het experiment overleefden toont aan dat er duidelijke verschillen zijn tussen sub-populaties met verschillende afkomst. Binnen de Amsterdamse sub-populatie wordt hoge genetische differentiatie gevonden. Dit is de eerste waarneming van een *M. leidyi* genotype dat zich bij zulke lage zoutgehaltes kan voortplanten. Ribkwallen van dit genotype zouden zich kunnen verspreiden naar gebieden waar tot nu toe van werd aangenomen dat het lage zoutgehalte de voortplanting van *M. leidyi* belemmerde.

In **hoofdstuk 8** is een kwalitatieve schatting gemaakt van voedselconcurrentie tussen *Mnemiopsis leidyi* en andere dierlijk plankton etende diersoorten in de Waddenzee. Aan de hand van Stabiele Isotopen Analyses (SIA) werd de overlap in dieet tussen vissen, kwalachtigen, kreeftachtigen en inktvissen onderzocht. Een clusteranalyse toonde aan dat gemiddelde δ^{13}C en δ^{15}N verhoudingen van *M. leidyi* hetzelfde waren als die van planktonetende vissoorten zoals de glasgrondel *Aphia minuta*, de haring *Clupea harengus* en de horsmakreel *Trachurus trachurus*. Ook

was er overlap met δ^{13}C en δ^{15}N verhoudingen van de kompaskwal *Chrysaora hysoscella*, de zeedruif *Pleurobrachia pileus* en de hydroidkwal *Nemopsis bachei*. δ^{15}N van *M. leidyi* was positief gerelateerd aan de groote van de ribkwallen, wat suggereert dat kleinere ribkwallen op een lager trofisch niveau zitten dan grote. Aan het begin van de bloeiperiode in augustus bestond vrijwel de hele *M. leidyi* populatie uit larven en juveniele ribkwallen en was er geen overlap met de stabiele isotopen niche van andere pelagische zooplankton eters. De meeste overlap tussen het dieet van *M. leidyi* men dat van andere soorten treedt op in perioden waarin de ribkwallen het minst talrijk zijn, wat suggereert dat *M. leidyi* op dit moment nog geen belangrijke voedselconcurrent is voor inheemse kwalachtigen en vissoorten. Tijdens de periode waarin *M. leidyi* het meest algemeen is, is de dichtheid aan concurrerende kwalachtigen laag. Dit suggereert dat *M. leidyi* een ongebruikte niche heeft opgevuld.

Nederlandse kwalachtigen in de toekomst

Dit proefschrift is een belangrijke aanvulling op onze kennis over de fenologie, het voorkomen en de soortensamenstelling van kwalachtigen in Nederlandse kustwateren. De ontwikkelde en geteste technieken zoals het Dynamisch Energie Budget (DEB) model, de fixatie- en preservatiemethode voor *Mnemiopsis leidyi* en het "common-garden" kweekexperiment kunnen ook toegepast worden bij onderzoek aan kwalachtigen in andere gebieden.

Het zou interessant zijn als het onderzoek naar identificatie en populatiestructuur van kwalpoliepen (**hoofdstuk 3**) wordt uitgebreid naar meer gebieden en habitat-types aangezien de locatie van poliepen van andere soorten dan *Aurelia aurita* nog steeds een mysterie is.

Toepassingen van het Dynamische Energie Budget (DEB) model geparametriseerd in **hoofdstuk 6** worden beschreven in **hoofdstuk 9**. Een ecosysteem model (GETM-ERSEM) laat zien dat *M. leidyi* ribkwallen vanuit de zuidelijke Noordzee naar de centrale Noordzee en Duitse Bocht getransporteerd worden. Aan de hand van de tijdens dit transport aanwezige omstandigheden kon met het DEB model de overleving en voortplanting van *M. leidyi* tijdens het transport voorspeld worden. Hieruit blijkt dat de Nederlandse wateren een bron van *M. leidyi* ribkwallen zijn voor meer noordelijk gelegen Europese wateren. Toepassing van het DEB model leverde nog enkele interessante hypotheses op. Grotere *M. leidyi* ribkwallen zijn gevoeliger voor afnames in het voedselaanbod dan kleinere maar kunnen veel meer eieren produceren onder gunstige omstandigheden. Bij lagere temperaturen en weinig voedsel lijken de ribkwallen het moment waarop zij geslachtsrijp worden uit te kunnen stellen, om zo lange perioden met een laag voedselaanbod te kunnen overleven. Deze hypotheses zouden experimenteel getest kunnen worden.

Concluderend laten de resultaten van dit proefschrift zien dat de introductie van *Mnemiopsis leidyi* in Nederlandse kustwateren op dit moment nog weinig impact heeft op de populaties van andere plankton-etende vissen en kwalachtigen. Echter, aangezien de voortplanting en groei van *Mnemiopsis leidyi* sterk afhankelijk is van de watertemperatuur zou opwarming van het water door klimaatverandering

verschuivingen in de seizoenspatronen van voorkomen van de ribkwallen kunnen veroorzaken. Daardoor kan de soort bloeien gaan vormen in jaargetijden waar nu nog temperatuur de limiterende factor is. Ook door de in dit proefschrift aangetoonde lagere tolerantiegrens voor zoutgehaltes kan de soort op plekken opduiken waar dit niet wordt verwacht. Op dit moment is monitoring van het dierlijk plankton in Nederlandse kustwateren helaas vrijwel afwezig waardoor bloeien van *M. leidyi* en de introductie van andere potentieel schadelijke soorten waarschijnlijk niet, of te laat, opgemerkt zullen worden.

About the author

Lodewijk van Walraven was born on 25 march 1986 in Warnsveld (Zutphen), the Netherlands. Through holidays on the Frisian isles and camps and excursions of the Dutch youth association for nature and environmental study (JNM) he became interested in marine biology at a young age. After attending pre-university college in Zutphen he studied Marine Biology at the University of Groningen. For his MSc thesis he studied fisheries induced evolution in North Sea plaice at Wageningen IMARES, and the seasonal pattern of the invasive ctenophore *Mnemiopsis leidyi* in the western Wadden Sea at NIOZ Royal Netherlands Institute for Sea Research. After working one year as a research assistant at Royal NIOZ he started the PhD that resulted in this thesis at the same institute. At the moment he works at Royal NIOZ on the impact of sustainable energy (Reverse Electro Dialysis, RED) on the marine environment, and as sustainable fisheries researcher at Good Fish Foundation.

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